

Fig. 1. A representation of the Fenton reaction and its role as a mediator in EMF-induced bioeffects.

were made. For example, in the study by Lagroye et al. [43] to investigate the effect of PK digestion on DNA migration after RFR exposure, PK was added to a lysing solution containing the detergent Triton X-100, which would inactivate the enzyme. Our experience indicates that the comet assay is a very sensitive and requires great care to perform. Thus, different detection sensitivities could result in different laboratories, even if the same procedures are followed. One way to solve this problem of experimental variation is for each research team to report the sensitivity of their comet assay, e.g., the threshold of detecting strand breaks in human lymphocytes exposed to X-rays. This information has generally not been provided for EMF-genotoxicity studies. Interestingly, when such information was provided, a large range of sensitivities have been reported. Malyapa et al. [40] reported a detection level of 0.6 cGy of gamma radiation in human lymphocytes, whereas McNamee et al. [76] reported 10–50 cGy of X-irradiation in lymphocytes, which is much higher than the generally acceptable detection level of the comet assay [15].

A drawback in the interpretation and understanding of experimental data from bioelectromagnetics research is that there is no general acceptable mechanism on how EMF affects biological systems. The mechanism by which EMF produces changes in DNA is unknown. Since the energy level associated with EMF exposure is not sufficient to cause direct breakage of chemical bonds within molecules, the effects are probably indirect and secondary to other induced biochemical changes in cells.

One possibility is that DNA is damaged by free radicals that are formed inside cells. Free radicals affect cells by damaging macromolecules, such as DNA, protein, and membrane lipids. Several reports have indicated that EMF enhances free radical activity in cells [18,19,61,62,77,78], particularly via the Fenton reaction [62]. The Fenton reaction is a process catalyzed by iron in which hydrogen peroxide, a product of oxidative respiration in the mitochondria, is converted into hydroxyl free radicals, which are very potent and cytotoxic molecules (Fig. 1).

It is interesting that ELF EMF has also been shown to cause DNA damage. Furthermore, free radicals have been implicated in this effect of ELF EMF. This further supports the view that EMF affects DNA via an indirect secondary process, since the energy content of ELF EMF is much lower than that of RFR. Effects via the Fenton reaction predict how a cell would respond to EMF. For instance:

- (1) Cells that are metabolically active would be more susceptible to EMF, because more hydrogen peroxide is generated by mitochondria to fuel the reaction.
- (2) Cells that have high level of intracellular free iron would be more vulnerable to EMF. Cancer cells and cells undergoing abnormal proliferation have higher concentrations of free iron because they uptake more iron and have less efficient iron storage regulation. Thus, these cells could be selectively damaged by EMF. Consequently, this suggests that EMF could potentially be used for the treatment of cancer and hyperplastic diseases. The effect could be further enhanced if one could shift anaerobic glycolysis of cancer cells to oxidative glycolysis. There is quite a large database of information on the effects of EMF (mostly in the ELF range) on cancer cells and tumors. The data tend to indicate that EMF could retard tumor growth and kill cancer cells. One consequence of this consideration is that epidemiological studies of cancer incidence in cell phone users may not show a risk at all or even a protection effect.
- (3) Since the brain is exposed to rather high levels of EMF during cell phone use, the consequences of EMF-induced genetic damage in brain cells are of particular importance. Brain cells have high levels of iron. Special molecular pumps are present on nerve cell nuclear membranes to pump iron into the nucleus. Iron atoms have been found to intercalate within DNA molecules. In addition, nerve cells have a low capacity for DNA repair, and DNA breaks could easily accumulate. Another concern is the presence of superparamagnetic iron-particles (magnetites) in body tissues, particularly in the brain. These particles could enhance free radical activity in cells and thus increase the cellular-damaging effects of EMF. These factors make nerve cells more vulnerable to EMF. Thus, the effect of EMF on DNA could conceivably be more significant on nerve cells than on other cell types of the body. Since nerve cells do not divide and are not likely to become cancerous, the more likely consequences of DNA damage in nerve cells include changes in cellular functions and in cell death, which could either lead to or accelerate the development of neurodegenerative diseases. Double-strand breaks, if not properly repaired, are known to lead to cell death. Cumulative DNA damage in nerve cells of the brain has been associated with neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases. However, another type of brain cell, the glial cell, can become cancerous as a result of DNA damage. The question is whether the damaged cells

would develop into tumors before they are killed by EMF due to over accumulation of genetic damages. The outcome depends on the interplay of these different physical and biological factors—an increase, decrease, or no significant change in cancer risk could result from EMF exposure.

- (4) On the other hand, cells with high amounts of antioxidants and antioxidative enzymes would be less susceptible to EMF. Furthermore, the effect of free radicals could depend on the nutritional status of an individual, e.g., availability of dietary antioxidants, consumption of alcohol, and amount of food consumption. Various life conditions, such as psychological stress and strenuous physical exercise, have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can also speculate that some individuals may be more susceptible to the effects of EMF exposure.

Additionally, the work of Blank and Soo [79] and Blank and Goodman [80] support the possibility that EMF exposure at low levels has a direct effect on electron transfer processes. Although the authors do not discuss their work in the context of EMF-induced DNA damage, the possibility exists that EMF exposure could produce oxidative damage to DNA.

## 5. Lessons learned

Whether or not EMF causes biological effects, let alone effects that are detrimental to human health and development, is a contentious issue. The literature in this area abounds with apparently contradictory studies, and as presented in this review, the literature specific to the effects of RFR exposure on DNA damage and repair in various biological systems is no exception. As a consequence of this controversy, there are several key issues that must be addressed—contrary data, weight of evidence, and data interpretation consistent with known science.

Consider that EMF does not share the familiar and comforting physical properties of chemical agents. EMF cannot be seen, tasted, smelled, or felt (except at high intensities). It is relevant, therefore, to ask, in what ways do scientists respond to data, especially if that data are contrary to their scientific beliefs or inconsistent with long-held hypotheses? Often such data are ignored, simply because it contradicts what is accepted as conventional wisdom. Careful evaluation and interpretation of data may be difficult, because technologies used to expose biological systems to EMF and methodologies used to assess dosimetry generally are outside the experience of most biomedical scientists. Additionally, it is often difficult to assess differences in methodologies between studies, one or more of which were intended to replicate an original investigation. For instance, Malyapa et al. [40] reported what they claimed to be a replication of the work of Lai and Singh [16]. There were, however, significant differences

in the comet analyses used by each group. Lai and Singh precipitated DNA in agarose so that low levels of DNA damage could be detected. Malyapa et al. did not. Lai and Singh treated their samples with PK to digest proteins bound to DNA, thus allowing DNA to move toward the positive pole during electrophoresis (unlike DNA, most proteins are negatively charged, and if they are not removed they will drag the DNA toward the negative pole). The Malyapa et al. study did not use PK. There were other methodological differences as well. Such is also the case in the study of Hook et al. [42], which attempted to replicate the work of Phillips et al. [21]. The latter group used a PK treatment in their comet assay, while the former group did not.

While credibility is enhanced when one can relate data to personal knowledge and scientific beliefs, it has not yet been determined how RFR couples with biological systems or by what mechanisms effects are produced. Even carefully designed and well executed RFR exposure studies may be summarily dismissed as methodologically unsound, or the data may be interpreted as invalid because of inconsistencies with what one believes to be correct. The quintessential example is the belief that exposure to RFR can produce no effects that are not related to the ability of RFR to produce heat, that is, to raise the temperature of biological systems [81,82]. Nonetheless, there are many examples of biological effects resulting from low-level (athermal) RFR exposure [83,84]. Consider here the work of Mashevich et al. [85]. This group exposed human peripheral blood lymphocytes to an 830-MHz signal for 72 h and at different average SARs (SAR, 1.6–8.8 W/kg). Temperatures ranged from 34.5 to 38.5 °C. This group observed an increase in chromosome 17 aneuploidy that varied linearly with SAR. Temperature elevation alone in the range of 34.5–38.5 °C did not produce this genotoxic effect, although significant aneuploidy was observed at higher temperatures of 40–41 °C. The authors conclude that the genotoxic effect of the radiofrequency signal used is elicited through a non-thermal pathway.

Also consider one aspect of the work of Phillips et al. [21]. In that study, DNA damage was found to vary in direction; that is, under some conditions of signal characteristics, signal intensity, and time of exposure, DNA damage increased as compared with concurrent unexposed controls, while under other conditions DNA damage decreased as compared with controls. The dual nature of Phillips et al.'s [21] results will be discussed later. For now consider the relationship of these results to other investigations. Adey et al. [86] performed an *in vivo* study to determine if rats treated *in utero* with the carcinogen ethylnitrosourea (ENU) and exposed to an 836.55-MHz field with North American Digital Cellular modulation (referred to as a TDMA field) would develop increased numbers of central system tumors. This group reported that rather than seeing an increase in tumor incidence in RFR-exposed rats, there was instead a decrease in tumor incidence. Moreover, rats that received no ENU but which were exposed to the TDMA signal also showed a decrease in the number of spontaneous tumors as compared

with animals exposed to neither ENU nor the TDMA signal. This group postulated that their results may be mechanistically similar to the work of another group. Stammberger et al. [87] had previously reported that rats treated *in utero* with ENU and then exposed to low doses of X-irradiation exhibited significantly reduced incidences of brain tumors in adult life. Stammberger and colleagues [87] hypothesized that low-level X-irradiation produced DNA damage that then induced the repair enzyme O<sup>6</sup>-alkylguanine-DNA alkyltransferase (AT). Numerous groups have since reported that X-irradiation does indeed induce AT activity (e.g., [88,89]). In this context, it is significant that Phillips et al. [21] found that cells exposed *in vitro* to a TDMA signal identical to that used in the study of Adey et al. [86] produced a decrease in DNA damage under specific conditions of intensity and time of exposure (lower intensity, longer time; higher intensity, shorter time). These results raise the intriguing possibility that the decrease in tumor incidence in the study of Adey et al. [86] and the decrease in DNA damage in the study of Phillips et al. [21] both may have been the result of induction of AT activity resulting from DNA damage produced by exposure to the TDMA signal. This remains to be investigated.

Because the issue of RFR-induced bioeffects is contentious, and because the issue is tried in courtrooms and various public forums, a term heard frequently is weight of evidence. This term generally is used to describe a method by which all scientific evidence related to a causal hypothesis is considered and evaluated. This process is used extensively in matters of regulation, policy, and the law, and it provides a means of weighing results across different modalities of evidence. When considering the effects of RFR exposure on DNA damage and repair, modalities of evidence include studies of cells and tissues from laboratory animals exposed *in vivo* to RFR, studies of cells from humans exposed to RFR *in vivo*, and studies of cells exposed *in vitro* to RFR. While weight of evidence is gaining favor with regulators [90], its application by scientists to decide matters of science is often of questionable value. One of the reasons for this is that there generally is no discussion or characterization of what weight of evidence actually means in the context in which it is used. Additionally, the distinction between weight of evidence and strength of evidence often is lacking or not defined, and differences in methodologies between investigators are not considered. Consequently, weight of evidence generally amounts to what Krinsky [90] refers to as a "seat-of-the-pants qualitative assessment." Krinsky points out that according to this view, weight of evidence is "a vague term that scientists use when they apply implicit, qualitative, and/or subjective criteria to evaluate a body of evidence." Such is the case in the reviews by Juutilainen and Lang [91] and Verschaeve and Maes [92]. There is little emphasis on a critical analysis of similarities and differences in biological systems used, exposure regimens, data produced, and investigator's interpretations and conclusions. Rather, there is greater emphasis on the number of publications either finding or not finding an effect of RFR exposure on some endpoint.

To some investigators, weight of evidence does indeed refer to the balance (or imbalance) between the number of studies producing apparently opposing results, without regard to critical experimental variables. While understanding the role these variables play in determining experimental outcome could provide remarkable insights into defining mechanisms by which RFR produced biological effects, few seem interested in or willing to delve deeply into the science.

A final lesson can be derived from a statement made by Gos et al. [93] referring to the work of Phillips et al. [21]. Gos and colleagues state, "The results in the latter study (Phillips et al., 1998) are puzzling and difficult to interpret, as no consistent increase or decrease in signal in the comet assay at various SARs or times of exposure was identified." This statement is pointed out because studies of the biological effects of exposure to electromagnetic fields at any frequency are often viewed as outside of or distinct from what many refer to as *mainstream science*. However, what has been perceived as an inconsistent effect is indeed consistent with the observations of bimodal effects reported in hundreds of peer-reviewed publications. These bimodal effects may be dependent on concentration of an agent, time of incubation with an agent, or some other parameter relating to the state of the system under investigation. For instance, treatment of B cells for a short time (30 min) with the protein kinase C activator phorbol 12,13-dibutyrate increased proliferative responses to anti-immunoglobulin antibody, whereas treatment for a longer period of time ( $\geq 3$  h) suppressed proliferation [94]. In a study of  $\kappa$ -opioid agonists on locomotor activity in mice, Kuzmin et al. [95] reported that higher, analgesic doses of  $\kappa$ -agonists reduced rearing, motility, and locomotion in non-habituated mice. In contrast, lower, subanalgesic doses increased motor activity in a time-dependent manner. Dierov et al. [96] observed a bimodal effect of all-trans-retinoic acid (RA) on cell cycle progression in lymphoid cells that was temporally related to the length of exposure to RA. A final example is found in the work of Rosenstein et al. [97]. This group found that the activity of melatonin on depolarization-induced calcium influx by hypothalamic synaptosomes from rats sacrificed late evening (2000 h) depended on melatonin preincubation time. A short preincubation time (10 min) stimulated uptake, while a longer preincubation (30 min) inhibited calcium uptake. These effects were also dependent on the time of day when the rats were sacrificed. Effects were maximal at 2000 h, minimal at 2400 h, and intermediate at 400 h. At 1000 h, only inhibitory effects of melatonin on calcium uptake were observed. These examples point out that what appears to be inconsistency may instead be real events related to and determined by the agents involved and the state of the biological system under investigation. The results of Phillips et al. [21] may be the result of signal modulation, signal intensity, time of exposure, or state of the cells. The results may indicate a bimodal effect, or they may, as the investigators suggest, represent time- and signal-dependant changes in the balance between damage and repair because of direct or indirect effects of RFR exposure on repair mechanisms.

## 6. Summary

Exposure of laboratory animals *in vivo* and of cultured cells *in vitro* to various radiofrequency signals has produced changes in DNA damage in some investigations and not in others. That many of the studies on both sides of this issue have been done well is encouraging from a scientific perspective. RFR exposure does indeed appear to affect DNA damage and repair, and the total body of available data contains clues as to conditions producing effects and methodologies to detect them. This view is in contrast to that of those who believe that studies unable to replicate the work of others are more credible than the original studies, that studies showing no effects cancel studies showing an effect, or that studies showing effects are not credible simply because we do not understand how those effects might occur. Some may be tempted to apply incorrectly the teachings of Sir Karl Popper, one of the great science philosophers of the 20th century. Popper proposed that many examples may lend support to an hypothesis, while only one negative instance is required to refute it [98]. While this holds most strongly for logical subjects, such as mathematics, it does not hold well for more complex biological phenomena that are influenced by stochastic factors. Each study to investigate RFR-induced DNA damage must be evaluated on its own merits, and then studies that both show effects and do not show effects must be carefully evaluated to define the relationship of experimental variables to experimental outcomes and to assess the value of experimental methodologies to detect and measure these outcomes (see Section 2).

The lack of a causal or proven mechanism(s) to explain RFR-induced effects on DNA damage and repair does not decrease the credibility of studies in the scientific literature that report effects of RFR exposure, because there are several plausible mechanisms of action that can account for the observed effects. The relationship between cigarette smoking and lung cancer was accepted long before a mechanism was established. This, however, occurred on the strength of epidemiologic data [99]. Fortunately, relevant epidemiologic data relating long-term cell phone use (>10 years) to central nervous system tumors are beginning to appear [84,100–102], and these data point to an increased risk of acoustic neuroma, glioma and parotid gland tumors.

One plausible mechanism for RFR-induced DNA damage is free radical damage. After finding that two free radical scavengers (melatonin and N-tert-butyl- $\alpha$ -phenylnitron) prevent RFR-induced DNA damage in rat brain cells, Lai and Singh [62] hypothesized that this damage resulted from free radical generation. Subsequently, other reports appeared that also suggested free radical formation as a result of RFR exposure [103–105]. Additionally, some investigators have reported that non-thermal exposure to RFR alters protein structure and function [106–109]. Scientists are familiar with molecules interacting with proteins through lock-and-key or induced-fit mechanisms. It is accepted that such interactions provide energy to change protein conformation and protein

function. Indeed, discussions of these principles are presented in introductory biology and biochemistry courses. Perhaps then it is possible that RFR exposure, in a manner similar to that of chemical agents, provides sufficient energy to alter the structure of proteins involved in DNA repair mechanisms to the extent that their function also is changed. This has not yet been investigated.

When scientists maintain their beliefs in the face of contrary data, two diametrically opposed situations may result. On the one hand, data are seen as either right or wrong and there is no discussion to resolve disparities. On the other hand, and as Francis Crick [110] has pointed out, scientists who hold theoretically opposed positions may engage in fruitful debate to enhance understanding of underlying principles and advance science in general. While the latter certainly is preferable, there are external factors involving economics and politics that keep this from happening. It is time to acknowledge this and embark on the path of fruitful discussion. Great scientific discoveries await.

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# Genotoxic effects of radiofrequency electromagnetic fields

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## Abstract

101 publications are exploited which have studied genotoxicity of radiofrequency electromagnetic fields (RF-EMF) *in vivo* and *in vitro*. Of these 49 report a genotoxic effect and 42 do not. In addition, 8 studies failed to detect an influence on the genetic material, but showed that RF-EMF enhanced the genotoxic action of other chemical or physical agents. The controversial results may in part be explained by the different cellular systems. Moreover, inconsistencies may depend from the variety of analytical methods being used, which differ considerably with respect to sensitivity and specificity. Taking altogether there is ample evidence that RF-EMF can alter the genetic material of exposed cells *in vivo* and *in vitro* and in more than one way. This genotoxic action may be mediated by microthermal effects in cellular structures, formation of free radicals, or an interaction with DNA-repair mechanisms.

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**Keywords:** Gene mutations; Cytogenetic effects; DNA fragmentation; Mechanisms of genotoxicity

## 1. Introduction

Alterations of genetic information in somatic cells are the key event in the process of carcinogenesis [1,2]. Consequently any agent, which has a genotoxic attribute is suspected also to be cancerogenic. This is the driving force behind the multitude of studies on genotoxicity of radiofrequency electromagnetic fields (RF-EMF), conducted so far. A total of 101 publications on genotoxicity studies of RF-EMF are exploited here, of which 49 report genotoxic effects, subsequently marked as GT(+) (Table 1), 43 do not (Table 2), and 9 find, that RF-EMF do not induce genotoxic events by itself but enhance the genotoxic action of other physical or chemical agents (Table 3). Thus, in contrast to several reviews in the past [3–6], it now became evident that non-thermal genotoxic effects of RF-EMF is convincingly demonstrated by a substantial number of published studies. The studies have been performed with a variety of different test systems – some studies used more than one test system – which will be assigned here to the three principle endpoints of a genotoxic action: (1) effect on chromosomes, (2) DNA fragmentation, and (3) gene mutations.

## 2. Effect on chromosomes

This group comprises the analysis of numerical or structural anomalies of metaphase chromosomes (CA), sister-chromatid-exchanges (SCEs), and formation of micronuclei (MN). Of the 21 studies using CA, 9 are CA-positive, 11 CA-negative, and 1 reports an RF-induced enhancement of genotoxicity by X-rays.<sup>1</sup> In general proliferating cells are required for the study of chromosomal effects, however, micronuclei have also been analysed in polychromatic erythrocytes and in exfoliated cells, for instance from buccal smears [7,8]. Moreover, aneuploidy rates of distinct chromosomes as well as chromosomal translocations can also be studied in interphase nuclei using fluorescence *in situ* hybridization (FISH). While structural aberrations detected by conventional CA are mainly lethal to the cell, translocations are persistent and may be passed to the cellular progeny. Using FISH increased levels of aneuploidy of chromosome 1, 10, 11, and 17 have been reported in human blood lymphocytes after RF-EMF exposure [9]. In metaphase chromosomes FISH may increase the sensitivity of chromosomal analysis [10] but this has only once been used for RF-EMF studies [11].

CA brings about to detect a variety of chromosomal aberrations. In contrast, micronuclei originate only from acentric

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Table 1  
Publications which report RF-EMF related genotoxic effects.

| Reference                 | Biological system                                      | Genotoxic endpoint  | Results and comments   |
|---------------------------|--|---|--|
| Aitken et al. [45]        | Mouse sperm  | QPCR and comet assay  | Gel electrophoresis revealed no gross evidence of increased single- or double-DNA strand breakage in spermatozoa. However, a detailed analysis of DNA integrity using QPCR revealed damage to both the mitochondrial genome ( $p < 0.05$ ) and the nuclear-globin locus ( $p < 0.01$ ).                    |
| Balode [46]               | Cow erythrocytes                                       | Micronuclei (MN)  | The counting of micronuclei in peripheral erythrocytes gave low average incidences, 0.6 per 1000 in the exposed group and 0.1 per 1000 in the control, but statistically significant ( $p < 0.01$ ) differences were found in the frequency distribution between the control and exposed groups.           |
| Belyaev et al. [47]       | Human blood lymphocytes                                | Chromatin condensation and 53BP1 foci   | Decrease in background levels of 53BP1 foci and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation.  |
| Busljeta et al. [48]      | Rat hematopoietic tissues                              | MN  | Erythrocyte count, haemoglobin and haematocrit were increased in peripheral blood (days 8 and 15). Concurrently, anuclear cells and erythropoietic precursor cells were decreased ( $p < 0.05$ ) in the bone marrow on day 15, but micronucleated cells' (MNCs) frequency was increased.                   |
| d'Ambrosio et al. [49]    | Human blood lymphocytes                                | MN  | The micronucleus frequency was not affected by CW exposure; however, a statistically significant micronucleus effect was found following exposure to phase modulated field.  |
| Diem et al. [23]          | Human cultured fibroblasts and rat granulosa cells     | Alkaline and neutral comet assay  | The intermittent exposure showed a stronger effect in the comet assay than continuous exposure.  |
| Ferreira et al. [50]      | Rat hematopoietic tissues exposed during embryogenesis | MN  | The irradiated group showed a significant increase in MN occurrence.   |
| Fucic et al. [15]         | Human blood lymphocytes                                | MN  | X-rays and microwaves were preferentially clastogens while vinyl chloride monomer showed aneugenic activity as well. Microwaves possess some mutagenic characteristics typical of chemical mutagens.   |
| Gadhia et al. [51]        | Human blood lymphocytes                                | Chromosomal aberrations and SCE   | There was a significant increase ( $p < 0.05$ ) in dicentric chromosomes among mobile users who were smoker-alcoholic as compared to nonsmoker-nonalcoholic. Synergistic action with MMC, SCEs showed a significant increase among mobile users.   |
| Gandhi and Singh [7]      | Human blood lymphocytes and buccal mucosa cells        | Chromosomal aberrations and MN  | Increased number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes.   |
| Gandhi, 2005 [52]         | Human blood lymphocytes                                | Comet assay, <i>in vivo</i> capillary MN  | Mean comet tail length ( $26.76 \pm 0.054$ mm; 39.75% of cells damaged) in mobile phone users was highly significant from that in the control group. The <i>in vivo</i> capillary blood MNT also revealed highly significant (0.25) frequency of micronucleated cells.                                     |
| Garaj-Vrhovac et al. [53] | Human blood lymphocytes                                | Chromosomal aberrations and MN  | In all experimental conditions, the frequency of all types of chromosomal aberrations was significantly higher than in the control samples. In the irradiated samples the presence of dicentric and ring chromosomes was established. The incidence of micronuclei was also higher in the exposed samples. |
| Garaj-Vrhovac et al. [54] | Chinese hamster cells V79                              | DNA synthesis by [ <sup>3</sup> H]thymidine uptake, and chromosomal aberrations | In comparison with the control samples there was a higher frequency of specific chromosome lesions in cells that had been irradiated.  |
| Garaj-Vrhovac et al. [55] | Chinese hamster cells V79                              | Chromosomal aberrations and MN  | Significantly higher frequency of specific chromosome aberrations such as dicentric and ring chromosomes in irradiated cells. The presence of micronuclei in irradiated cells confirmed the changes that had occurred in chromosome structure.   |
| Garaj-Vrhovac et al. [56] | Human blood lymphocytes                                | MN  | Increase in frequency of micronuclei as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls.  |
| Haider et al. [57]        | <i>Tradescantia</i> flower buds                        | MN  | The results at all exposure sites except one were statistically significant.   |
| Koyama et al. [12]        | CHO-K1 cells   | MN + kinetochore determination  | RF at SAR of 78 W/kg and higher form MN with a particular increase of kinetochore-positive MN and potentiate MN formation induced by bleomycine treatment.   |
| Lai et al. [58]           | Rat brain cells  | Comet assay   | RFR exposure significantly increased DNA double strand breaks in brain cells of the rat, and the effect was partially blocked by treatment with naltrexone.  |
| Lai and Singh [59]        | Rat brain cells  | Alkaline comet assay  | No effects immediately after 2 h of exposure to pulsed microwaves, whereas a dose rate-dependent increase in DNA single strand breaks was found in brain cells of rats at 4 h post-exposure with CW and pulsed waves.  |

|                         |   |  |  |
|-------------------------|---|--|--|
| Lai and Singh [60]      | Rat brain cells                               | Comet assay  | Significantly higher levels of DNA single and double strand breaks. Exposure to 'noise' alone did not significantly affect the levels, however, simultaneous 'noise' exposure blocked microwave-induced increases in DNA strand breaks.  |
| Lai and Singh [61]      | Rat brain cells                               | Comet assay  | An increase in DNA strand breaks was observed after exposure to either the pulsed or continuous-wave radiation, no significant difference was observed between the effects of the two forms of radiation.  |
| Lai and Singh [35]      | Rat brain cells                               | Comet assay  | Treatment immediately before and after RFR exposure with either melatonin or <i>N</i> -tert-butyl- $\alpha$ -phenylnitron (PBN) blocks induction of DSB by RFR. It is hypothesized that free radicals are involved in RFR-induced DNA damage in the brain cells of rats.   |
| Lixia et al. [62]       | Human lens epithelial cells                   | Comet assay and BudR incorporation   | No DNA breaks at 1 and 2 W/kg but increase 0 and 30 min after exposure to 3 W/kg. Exposure at 2 and 3 W/kg for 2 h significantly increased HsP 70 protein but not mRNA expression.   |
| Maes et al. [63]        | Human blood lymphocytes                       | Chromosome aberrations   | Some cytogenetic damage was obtained <i>in vitro</i> when blood samples were very close to the antenna. The questionable <i>in vivo</i> results (six maintenance workers) are not considered here.   |
| Maes et al. [64]        | Human blood lymphocytes                       | Chromosomal aberrations, SCE, and MN   | Marked increase in the frequency of chromosome aberrations (including dicentric chromosomes and acentric fragments) and 19 micronuclei. On the other hand, the microwave exposure did not influence the cell kinetics nor the sister-chromatid-exchange (SCE) frequency.   |
| Markova et al. [65]     | Human blood lymphocytes                       | p53 binding protein and $\gamma$ H2AX foci                                   | MWs from GSM mobile telephones affect chromatin conformation and 53BP1/ $\gamma$ H2AX foci similar to heat shock.  |
| Mashevich et al. [66]   | Human blood lymphocytes                       | Chromosomal aberrations  | A linear increase in chromosome 17 aneuploidy was observed as a function of the SAR value.   |
| Mazor et al. [9]        | Human blood lymphocytes                       | Aneuploidy rate of Chr. # 1, 10, 11, 17 determined by interphase FISH        | Increased levels of aneuploidy in chromosomes 1 and 10 at higher SAR, while for chromosomes 11 and 17 the increases were observed only for the lower SAR.  |
| Nikolova et al. [67]    | Mouse nestin-positive neural progenitor cells | Transcript of specific genes and proteins, proliferation, apoptosis, DNA DSB | Down-regulation of neural-specific Nurr1 and up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double strand breaks.  |
| Paulraj and Behari [68] | Rat brain cells                               | Comet assay  | Statistically significant ( $p < 0.001$ ) increase in DNA single strand breaks in brain cells of rat.  |
| Pavicic and Trosic [13] | V79 cells                                     | Alteration of microtubule proteins   | The microtubule structure altered after 3 h of irritation.   |
| Phillips et al. [69]    | Molt-4 T-lymphoblastoid cells                 | Comet assay  | DNA damage decreased by (1) exposure to the iDEN signal (2.4 $\mu$ W/g for 2 h or 21 h), (2) exposure to the TDMA signal (2.6 $\mu$ W/g for 2 h and 21 h), (3) exposure to the TDMA signal (26 $\mu$ W/g for 2 h), exposure to the iDEN signal (24 $\mu$ W/g for 2 h) and 21 h significantly increased DNA damage. |
| Sarimov et al. [70]     | Human blood lymphocytes                       | Chromatin condensation by anomalous viscosity                                | Analysis of pooled data from all donors showed statistically significant effect of 1-h exposure to MW. Effects differ at various GSM frequencies and vary between donors.  |
| Sarkar et al. [71]      | Mouse testis and brain cells                  | Restriction pattern after HinfI treatment                                    | As compared to control animals, band patterns in exposed animals were found to be distinctly altered in the range of 7–8 kb which was also substantiated by densitometric analysis.  |
| Schwarz et al. [33]     | Human cultured fibroblasts and lymphocytes    | Alkaline comet assay and MN  | UMTS exposure increased the CTF and induced centromere-negative micronuclei in human cultured fibroblasts in a dose- and time-dependent way. No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with phytohemagglutinin.  |
| Sykes et al. [22]       | pKZ1 mice                                     | lacZ transgene inversion   | No difference between the control and treated groups in the 1- and 5-day exposure groups, but a reduction in inversions below the spontaneous frequency in the 25-day exposure group. This suggests that RF radiation can lead to a perturbation in recombination frequency.                                       |
| Tice et al. [72]        | Human blood lymphocytes                       | Alkaline comet assay and MN  | Exposure for either 3 or 24 h with the unmodulated signal did not induce a significant increase in DNA DSB or MN in lymphocytes. However, with the modulated signal there was a significant and reproducible increase in the frequency of micronucleated lymphocytes.  |
| Tkalec et al. [14]      | <i>Allium cepa</i> seeds                      | Germination, mitotic index, mitotic abnormalities                            | Increased mitotic aberrations in root meristematic cells of <i>A. cepa</i> . Effects were markedly dependent on the field frequencies applied as well as on field strength and modulation. Findings also indicate that mitotic effects of RF-EMF could be due to impairment of the mitotic spindle.                |
| Trosic et al. [73]      | Rat hematopoietic tissues                     | MN and polychromatic erythrocytes (PCEs)                                     | The incidence of micronuclei/1000 PCEs in peripheral blood was significantly increased ( $p < 0.05$ ) in the subgroup exposed to fr/MW radiation after eight irradiation treatments of 2 h each in comparison with the sham-exposed control group.   |

Table 1 (Continued)

| Reference                  | Biological system   | Genotoxic endpoint                                  | Results and comments   |
|----------------------------|---|---|--|
| Trosic et al. [74]         | Rat hematopoietic tissues                                   | MN and polychromatic erythrocytes                   | In polychromatic erythrocytes significant differences ( $p < 0.05$ ) for experimental days 8 and 15. The frequency of micronucleated PCEs was also significantly increased on experimental day 15 ( $p < 0.05$ ).  |
| Trosic and Busljeta [75]   | Rat hematopoietic tissues and peripheral blood              | MN and polychromatic erythrocytes                   | BMPCEs were increased on days 8 and 15, and PBPCs were elevated on days 2 and 8 ( $p < 0.05$ ).  |
| Vijayalaxmi et al. [76]    | C3H/HeJ cancer prone mice, peripheral blood and bone marrow | MN  | No observed RF effects. A correction was published, stating that there was actually a significant MN increase in peripheral blood and bone marrow cells after chronic exposure to RF [Vijayalaxmi, M.R. Frei, S.J. Dusch, V. Guel, M.L. Meltz, J.R. Jauchem, Radiat. Res. 149 (3) (1998) 308].                                 |
| Wu et al. [39]             | Human epithelial lens cells                                 | Comet assay and intracellular ROS                   | RF at 4 W/kg for 24 h significantly increased intracellular ROS and DNA damage. Both can be blocked completely by electromagnetic noise.   |
| Yadav and Sharma [8]       | Exfoliated buccal cells                                     | MN in buccal cells                                  | In exposed subjects $9.84 \pm 0.745$ micronucleated cells and $10.72 \pm 0.889$ total micronuclei (TMN) as compared to zero duration of exposure along with average $3.75 \pm 0.774$ MNC and $4.00 \pm 0.808$ TMN in controls. Correlation between 0–1, 1–2, 2–3 and 3–4 years of exposure and the frequency of MNC and TMN.   |
| Yao et al. [40]            | Human lens epithelial cells                                 | Alkaline comet assay, gamma-H2AX foci, ROS level    | SAR of 3 and 4 W/kg induced significant DNA damage in the comet assay, while no statistical difference in double strand breaks was found by $\gamma$ H2AX foci. Electromagnetic noise could block RF-induced ROS formation and DNA damage.   |
| Yao et al. [41]            | Human lens epithelial cells                                 | Alkaline comet assay, $\gamma$ H2AX foci, ROS level | DNA damage was significantly increased by comet assay at 3 and 4 W/kg, whereas double strand breaks by $\gamma$ H2AX foci were significantly increased only at 4 W/kg. Significantly increased ROS levels were detected in the 3 and 4 W/kg groups.  |
| Zhang et al. [77]          | Chinese hamster lung cells (CHL)                            | $\gamma$ H2AX foci                                  | Increased percentage of $\gamma$ H2AX foci positive cell of 1800 MHz RF EMF exposure for 24 h ( $37.9 \pm 8.6\%$ ) or 2-acetylaminofluorene exposure ( $50.9 \pm 9.4\%$ ). However, there was no significant difference between the sham-exposure and RF EMF exposure for 1 h ( $31.8 \pm 8.7\%$ ).                            |
| Zotti-Martelli et al. [78] | Human blood lymphocytes                                     | MN  | Both spontaneous and induced MN frequencies varied in a highly significant way among donors ( $p < 0.009$ ) and between experiments ( $p < 0.002$ ), and a statistically significant increase of MN, although rather low, was observed dependent on exposure time ( $p = 0.0004$ ) and applied power density ( $p = 0.0166$ ). |
| Zotti-Martelli et al. [79] | Human blood lymphocytes                                     | MN  | The results showed for both radiation frequencies an induction of micronuclei as compared to the control cultures at a power density of $30 \text{ mW/cm}^2$ and after an exposure of 30 and 60 min.   |

Abbreviations: Mitomycin C (MMC), bleomycin (BLM), methylmethanesulfonate (MMS), 4-nitroquinoline-1-oxide (4-NQ1O), ethylmethanesulfonate (EMS), chromosomal aberration analysis (CA), micronucleus assay (MN), reactive oxygen species (ROS), and fluorescence *in vitro* hybridization (FISH).

Table 2

Publications which do not report RF-EMF related genotoxic effects.

| Reference                | Biological system  | Genotoxic endpoint   | Results and comments  |
|--------------------------|--|--|---|
| Antonopoulos et al. [80] | Human blood lymphocytes                                  | SCE  | No increase in SCE or cell cycle progression found.   |
| Belyaev et al. [81]      | Rat brain, spleen, and thymus                            | Comet assay  | GSM MWs at 915 MHz did not induce PFGE-detectable DNA double stranded breaks or changes in chromatin conformation, but affected expression of genes in rat brain cells.   |
| Bisht et al. [82]        | Mouse C3H 10T cells                                      | MN   | CDMA (3.2 or 4.8 W/kg) or FDMA (3.2 or 5.1 W/kg) RF-EMF radiation for 3, 8, 16 or 24 h did not result in a significant increase either in the percentage of binucleated cells with micronuclei or in the number of micronuclei per 100 binucleated cells. |
| Chang et al. [83]        | <i>Escherichia coli</i> tester strain                    | Bacterial mutagenicity (Ames test)                           | No mutagenic or co-mutagenic effect with 4-NQ10.  |
| Ciaravino et al. [84]    | CHO cells  | SCE  | Radiofrequency electromagnetic radiation (RF-EMF) did not change the number of SCEs that were induced by adriamycin.  |
| Garson et al. [85]       | Human blood lymphocytes                                  | CA   | No RF-EMF effect observed.  |
| Gorlitz et al. [86]      | B6C3F1 mice lymphocytes, erythrocytes, and keratinocytes | MN   | No visible effect.  |
| Gos et al. [87]          | <i>Saccharomyces cerevisiae</i>                          | Mutation rates   | No effects in fluctuation tests on forward mutation rates at CAN1, on the frequency of petite formation, on rates of intra-chromosomal deletion formation, or on rates of intra-genic recombination in the absence or presence of MMS.                    |
| Hook et al. [88]         | Molt-4 T lymphoblastoid cells                            | Comet assay  | No RF-EMF effects observed.   |
| Juutilainen et al. [89]  | Female CBA/S mice and K2 female transgenic mice          | MN in erythrocytes   | No effect on MN frequency.  |
| Kerbacher et al. [90]    | CHO cells  | CA   | No alteration was observed in the extent of chromosome aberrations induced by either simultaneous fro radiation exposure or convection heating to equivalent temperatures.  |
| Komatsubara et al. [91]  | Mouse m5S cells  | CA   | No effect on CA; temperature increase up to 41 °C at 100 W/kg.  |
| Koyama et al. [92]       | CHO cells  | MN   | No MN increase in cells exposed to HFEMF at a SAR of lower than 50 W/kg, while those at SARs of 100 and 200 W/kg were significantly higher when compared with the sham-exposed controls (temperature effect).   |
| Lagroye et al. [93]      | Rat brain cells  | Alkaline comet assay   | No observed effect.   |
| Lagroye et al. [94]      | C3H 10T1/2 cells   | Comet assay, DNA-protein crosslinks                          | No observed effect.   |
| Li et al. [95]           | Murine C3H 10T cells                                     | Comet assay  | No observed effect.   |
| Maes et al. [96]         | Human blood lymphocytes                                  | CA, SCE  | Combined exposure of RF-EMF and to MMC and X-rays. Overall, no indication was found of a mutagenic, and/or co-mutagenic/synergistic effect.   |
| Maes et al. [97]         | Human blood lymphocytes                                  | CA, SCE  | Combined treatments with X-rays or MMC did not provide any indication of a synergistic action between the RF-EMF fields and X-rays or MMC.  |
| Maes et al. [98]         | Human blood lymphocytes                                  | CA, SCE, Comet assay   | The alkaline comet assay, SCE, and CA tests revealed no evidence of RF-EMF-induced genetic effects. No cooperative action was found between the electromagnetic field exposure and MMC using either the comet assay or SCE test.                          |
| Malyapa et al. [99]      | Rat brain cells  | Comet assay  | No significant differences observed.  |
| Malyapa et al. [100]     | U87MG and C3H 10T1/2 cells                               | Comet assay  | No significant differences observed.  |
| Malyapa et al. [101]     | U87MG and C3H 10T1/2 cells                               | Comet assay  | No significant differences observed.  |
| McNamee et al. [102]     | Human blood lymphocytes                                  | Comet assay and MN   | No significant differences observed.  |
| McNamee et al. [103]     | Human blood lymphocytes                                  | Comet assay and MN   | No significant differences observed.  |
| McNamee et al. [104]     | Human blood lymphocytes                                  | Comet assay  | No significant differences observed.  |
| Meltz et al. [105]       | L5178Y mouse leukemic cells                              | Mutation in TK locus   | No effect of RF-EMF alone or in the induced mutant frequency due to the simultaneous exposure to RF-EMF and proclim, as compared with the proflavin exposures alone.  |
| Ono et al. [106]         | lacZ-transgenic mice                                     | Mutations at the lac gene in spleen, liver, brain and testis | Mutation frequencies at the lacZ gene in spleen, liver, brain, and testis were similar to those observed in non-exposed mice.   |

Table 2 (Continued)

| Reference                | Biological system  | Genotoxic endpoint                | Results and comments   |
|--------------------------|--|-----------------------------------|--|
| Roti Roti et al. [107]   | C3H 10T1/2 cells   | Transformed foci                  | No statistically significant differences observed.   |
| Sakuma et al. [108]      | Human glioblastoma A172 cells and fetal lung fibroblasts | DNA strand breaks (comet assay?)  | No statistically significant differences.  |
| Scarf et al. [109]       | Human blood lymphocytes                                  | MN                                | No statistically significant differences observed.   |
| Speit et al. [24]        | Human cultured fibroblasts                               | Comet assay and MN                | No statistically significant differences observed.   |
| Stronati et al. [110]    | Human blood lymphocytes                                  | Comet assay, CA, SCE, MN          | By comparison with appropriate sham-exposed and control samples, no effect of RF-EMF alone could be found for any of the assay endpoints. In addition RF-EMF did not modify any measured effects of the X-radiation. |
| Takahashi et al. [111]   | Big Blue mice brain tissues                              | lacZ transgene inversion          | No statistically significant differences observed.   |
| Verschaeve et al. [112]  | Rat brain and liver tissues, erythrocytes                | MN (erythrocytes) and comet assay | No genotoxic effect of RF-EMF alone. Co-exposures to MX and RF-EMF radiation did not significantly increase the response of blood, liver and brain cells compared to MX exposure only.                               |
| Vijayalaxmi et al. [113] | Human blood lymphocytes                                  | CA and MN                         | No observed RF-EMF effects.  |
| Vijayalaxmi et al. [114] | Human blood lymphocytes                                  | CA and MN                         | No observed RF-EMF effects.  |
| Vijayalaxmi et al. [115] | Human blood lymphocytes                                  | Comet assay                       | No observed RF-EMF effects.  |
| Vijayalaxmi et al. [116] | Human blood lymphocytes                                  | CA, MN                            | No observed RF-EMF effects.  |
| Vijayalaxmi et al. [117] | Rat hematopoietic tissues and erythrocytes               | MN                                | No observed RF-EMF effects.  |
| Vijayalaxmi et al. [118] | Rat whole body and head only exposures. BM erythrocytes  | MN                                | No observed RF-EMF effects.  |
| Vijayalaxmi et al. [119] | CF-1 male mice, peripheral blood and bone marrow         | MN                                | No observed RF-EMF effects.  |
| Zeni et al. [120]        | Human blood lymphocytes                                  | Comet assay, CA, SCE              | No observed RF-EMF effects.  |
| Zeni et al. [121]        | Human blood lymphocytes                                  | MN                                | No observed RF-EMF effects.  |

Abbreviations: Chromosomal aberration analysis (CA), methotrexat (MX), mitomycin C (MMC), 4-nitroquinoline-1-oxide (4-NQ1O), methylmethanesulfonate (MMS), code division multiple access (CDMA), frequency division multiple access (FDMA), and time division multiple access (TDMA).

Table 3  
Publications which report synergistic RF-EMF effects in combination with other genotoxins.

| Reference            | Genotoxic agents              | Biological system  | Genotoxic endpoint              | Results and comments  |
|----------------------|-------------------------------|--|---------------------------------|---|
| Baohong et al. [122] | MMC, BLM, MMS, 4-NQ10         | Human blood lymphocytes                                      | Alkaline comet assay            | 1.8 GHz RFR (SAR: 3 W/kg) for 2 h did not induce DSB, but could enhance the human lymphocyte DNA damage effects induced by MMC and 4-NQ10. The synergistic DNA damage effects with BLM or MMS were not obvious.                   |
| Baohong et al. [123] | 254 nm UVC                    | Human blood lymphocytes                                      | Alkaline comet assay            | RF exposure for 1.5 and 4 h did not enhance significantly human lymphocyte DNA damage, but could reduce and increase DNA damage of human lymphocytes induced by UVC at 1.5 and 4 h incubation respectively.                       |
| Kim et al. [124]     | Cyclophosphamide, 4-NQ10, EMS | L5178Y mouse lymphoma cells (comet assay) and CHL cells (CA) | Alkaline comet assay and CA     | No direct cytogenetic effect of RF alone or in combination with cyclophosphamide or 4-NQ10 was found in the CA test and in the comet assay. However, RF had a potentiating effect in combination with cyclophosphamide or 4-NQ10. |
| Maes et al. [125]    | MMC                           | Human blood lymphocytes                                      | SCE                             | Synergistic effect was observed with MMC.   |
| Maes et al. [126]    | MMC                           | Human blood lymphocytes                                      | CA, SCE, comet assay            | The combined exposure of the cells to the radiofrequency fields followed by their cultivation in the presence of mitomycin C revealed a very weak effect when compared to cells exposed to mitomycin C alone.                     |
| Manti et al. [11]    | Previous 4 Gy X-ray radiation | Human blood lymphocytes                                      | Chromosome aberration by FISH   | No significant variations due to the UMTS exposure in the fraction of aberrant cells, but frequency of exchanges per cell in X-ray irradiated cells was significantly increased by UMTS at 2 W/kg.                                |
| Wang et al. [127]    | 254 nm UVC                    | Human blood lymphocytes                                      | Comet assay                     | RF did not induce DNA damage but reduced or enhanced DNA damage by UVC at 1.5 or 4.0 h respectively.  |
| Wang et al. [128]    | MMC, BLM, MMS, 4-NQ10         | Human blood lymphocytes                                      | Comet assay                     | RF did not induce DNA damage but enhanced DNA damage induced by MMC and 4-NQ10.   |
| Zhang et al. [129]   | MMC                           | Human blood lymphocytes                                      | Comet assay, micronucleus assay | No RF-induced DNA and chromosome damage, but increased MMC DNA damage by RF in comet assay.   |

Abbreviations: Mitomycin C (MMC), bleomycin (BLM), methylmethanesulfonate (MMS), 4-nitroquinoline-1-oxide (4-NQ10), ethylmethanesulfonate (EMS), chromosomal aberration analysis (CA), fluorescence *in vitro* hybridization (FISH).

fragments of chromosomes or from lagged chromosomes secondary to mitotic non-disjunction, the latter being detected by indirect immunofluorescence using kinetochore antibodies. Kinetochore-positive MN arise by epigenetic mechanisms (disturbances of the spindle apparatus). Kinetochore-negative MN arise from acentric chromosomal fragments. This is an important distinction, but has been performed in a few RF-EMF studies only, of which only one [12] reports an increase of kinetochore-positive MN albeit after a high SAR  $\geq 78$  W/kg. Two studies describe RF-EMF-induced disturbances of the spindle apparatus [13,14], and one reports an aneugenic RF-EMF effect on the basis of the size distribution of MN [15]. Of a total of 39 studies using the micronucleus assay 22 are MN-positive, and 17 MN-negative.

SCEs are analysed in metaphase chromosomes after two rounds of replication in the presence of 5-bromodeoxyuridine (BUDR). SCEs, which are induced during the S-phase of the cell cycle, represent an exchange between homologous chromatids, an event which by itself is genetically neutral. Nevertheless it is considered to reflect a recombinational repair of DNA double strand breaks (DSB), and may therefore serve as an indicator of genotoxic stress. Of 10 studies using SCE a GT(+) effect was reported in one only, 8 were negative, and one study reports RF-induced enhancement of genotoxicity by mitomycin C.

### 3. DNA fragmentation

The comet assay, also known as a "Single Cell Gel electrophoresis assay" (SCG), and the detection of gamma-H2AX foci are the most frequently used techniques to study RF-EMF-induced DNA strand breaks. The comet assay uses interphase nuclear DNA, which is unwinded under alkaline conditions and subsequently subjected to an electric field. Here DNA fragments migrate towards the anode, thereby forming a comet-like tail [16,17]. The alkaline comet assay detects DNA single strand as well as double strand breaks, but is not applicable in the presence of DNA crosslinking agents [18]. These breaks may occur not only by toxic influences but also by transcriptional and repair processes and by alkali-sensitive sites. Therefore this frequently used and very sensitive assay has a poor specificity. Of 41 studies using the comet assay 15 report comet-positive and 19 comet-negative results after RF-EMF exposure. RF-EMF enhancement of comet assay effects caused by other genotoxic agents is described in 7 studies.

Out of a multitude of DNA damage checkpoint proteins two have been used to detect DSB: H2AX, a member of the nuclear histone family [19], and P53 binding protein (53BP1). Both are rapidly phosphorylated only minutes after DNA damage and are then gathered in the vicinity of DNA double strand breaks. Here they form foci which can be visualized by indirect immunofluorescence [20,21]. These foci represent an initial and specific step in the repair process of exogenously induced DNA double strand breaks. It is important to real-

ize, however, that repair processes of DSB are quantified, not DSB themselves. The method has been employed in 4 studies, predominantly using the  $\gamma$ H2AX foci test. In all instances GT(+) effects have been detected.

DNA alterations have also been analysed by the anomalous viscosity time dependency test (AVTD, 1 GT(+) study), detecting conformational changes, and by quantitative PCR (QPCR, 1 GT(+) study) detecting structural changes in the DNA.

### 4. Gene mutations

In this category 6 studies have been performed using 4 different endpoints: (1) Altered restriction fragments (1 GT(+) study), (2) lacZ inversion in transgenic mice. This method has been used in 3 studies which all failed to detect an increased rate of inversions, but one found a reduced rate as compared to unexposed controls [22], which is interpreted as a RF-EMF-induced reduction of recombination repair. (3) Mutation at the thymidine kinase (TK) locus (1 negative study). (4) Bacterial his<sup>-</sup> revertants (Ames test, 1 negative study).

### 5. Discussion

The large number of contradictory results among the 101 published studies on a genotoxic action of RF-EMF is tangle. Nevertheless patterns can be perceived. GT(+) as well as GT(-) findings have been reported at a standard absorption ratio (SAR) below 0.05 up to 100 W/kg and an exposure of 15 min and 48 h *in vitro*, and between hours and years *in vivo*. The outcome of studies was nearly independent from RF frequencies between 300 and 7700 MHz and the type of RF signal, either continuous wave (CW) or pulse-modulated (PM). GT(+) was obtained in 15 CW and 26 PM exposures, GT(-) in 14 CW and 27 PM exposures (some studies did not indicate the type of signal used). Contradictory results have been obtained even when two experienced groups performed the same experiments using the same cells and identical exposure conditions [23,24]. This may reflect a general problem of genotoxic studies being dependent on a multitude of factors which are difficult to control [25]. Some of the studies exploited here have shortcomings with respect to incompletely described or unreliable exposure conditions and/or an inadequate experimental design. Even a considerable publication bias in favour of negative results has been suspected ([www.microwavenews.com/RR.html](http://www.microwavenews.com/RR.html), 2006) [26].

The proportion of GT(+) effects is much higher *in vivo* (23/40) than *in vitro* (29/77). (Since some studies have been performed on more than one biological system, the total number of GT(+) and GT(-) effects exceeds the total number of published studies.) Considering all genotoxic endpoints applied, the frequently used parameters chromosome analysis (9/21 GT(+)), comet assay (15/41 GT(+)), and sister-chromatid-exchange (1/10 GT(+)) showed the highest

proportion of negative results, while the micronucleus assay yielded more positive than negative results (22/39 GT(+)). Since the SCE test which was negative in nearly all cases is known to be rather insensitive to radiomimetic (clastogenic) agents it can be speculated, that a clastogenic mechanism is involved in RF-EMF genotoxic action.

Epigenetic influences may also contribute to genotoxicity as demonstrated by RF-EMF-induced chromosomal non-disjunction and disturbances of the mitotic spindle. This is in agreement with the higher proportion of 22/39 GT(+) findings among studies using the micronucleus assay as compared to those using CA, because some of the micronuclei may represent lagged chromosomes. Epigenetic mechanisms may also be effective after a combined exposure to RF-EMF and various physical or chemical mutagens (Table 4). RF-EMF preferentially enhanced the genotoxic effect of 4-NQ10 (4/4), MMC (4/8), UVC (2/2), and cyclophosphamide (2/2). No synergistic effect was obtained using MMS and EMS (3/3), BLM (2/2), and adriamycin (2/2). Only one out of 3 studies reported a synergistic effect with X-rays.

Cells and tissues of different origin exhibit a clearly variable sensitivity for genotoxic RF-EMF effects (Table 4). This has also been observed with extremely low frequency (ELF)-EMF [27] and may be dependent on genetic differences [28]. GT(+) effects of RF-EMF were reported predominantly in the following biological systems: human lens epithelial cells (4/4), human buccal mucosa cells (2/2), rodent brain tissues (8/13), and rat hemopoietic tissues (5/7). GT(–) results have been obtained with mouse permanent cell lines (7/7) and

permanent lymphoblastoid cells of various origin (7/7). This is in a striking analogy to RF-EMF-induced reduction of ornithine decarboxylase activity being detected in primary but not in secondary neural cells [29].

## 6. Proposed mechanisms of RF-EMF genotoxicity

Cells are unusually sensitive to electromagnetic fields [30]. Weak fields may accelerate electron transfer and thereby destabilize the H-bond of cellular macromolecules. This could explain the stimulation of transcription and protein expression, which has been observed after RF-EMF exposure [31,32]. However, the energy of weak EM fields is not sufficient directly to break a chemical bond in DNA. Therefore it can be concluded, that genotoxic effects are mediated by indirect mechanisms as microthermal processes, generation of oxygen radicals (ROS), or a disturbance of DNA-repair processes.

### 6.1. Thermal effects

An increase of temperature in the culture medium of RF-EMF exposed cells has been observed at very high SAR levels only [12]. The vast majority of GT(+) studies were conducted at SAR < 2.0 not leading to a detectable increase of temperature in the culture medium. Moreover, similar or larger effects have been observed at a 5' on/10' off intermittent exposure [23,33], a result that contradicts a

Table 4  
Distribution RF-EMF effects in 101 published studies.

| Biological system                        | RF-EMF effects |          | Synergistic effects |          |
|--|----------------|----------|---------------------|----------|
|  | Positive       | Negative | Positive            | Negative |
| <i>In vitro</i> (all cells and tissues)  | 29             | 39       | 9                   | 11       |
| Human blood lymphocytes                  | 18             | 23       | 8                   | 4        |
| Human lens epithelial cells              | 4              |          |                     |          |
| Human cultured fibroblasts               | 2              | 2        |                     |          |
| Human glioblastoma cells                 |                | 3        |                     |          |
| Human lymphoblastoid cells               |                | 2        |                     |          |
| Mouse permanent cell lines               |                | 6        |                     | 1        |
| Mouse lymphoblastoid cells               |                | 1        | 1                   | 1        |
| Chinese hamster cells (CHO, V79)         | 4              | 2        |                     | 3        |
| <i>E. coli</i>                           |                | 1        |                     | 2        |
| Yeast                                    |                | 1        |                     |          |
| Rat granulosa cells                      | 1              |          |                     |          |
| <i>In vivo</i> (all species and tissues) | 23             | 17       | 0                   | 1        |
| Human blood lymphocytes                  | 4              | 2        |                     |          |
| Human buccal mucosa cells                | 2              |          |                     |          |
| Mouse sperm                              | 1              |          |                     |          |
| Mouse brain tissues                      | 2              |          |                     |          |
| Mouse polychromatic erythrocytes         |                | 4        |                     |          |
| Rat brain tissues                        | 6              | 4        |                     | 1        |
| Rat hemopoietic tissues                  | 5              | 2        |                     |          |
| Rat spleen, liver                        |                | 2        |                     |          |
| lacZ-transgenic mice                     |                | 3        |                     |          |
| Plants                                   | 2              |          |                     |          |
| Cattle polychromatic erythrocytes        | 1              |          |                     |          |

Since several published studies have used more than 1 biological system the total of negative and positive effects exceeds the number of 101 publications.

simple temperature-based mechanism of the observed genotoxic action. However, experimental results with microwave absorption at colloidal interfaces have demonstrated that the electric absorption of microwaves between 10 and 4000 MHz goes through a maximum with the size of bridge droplets >100 and <10,000 nm, and depends on the type of ions and their concentrations [34]. This local absorption of microwaves may therefore lead to a considerable local heating in living cells during low energy microwave exposure.

### 6.2. Oxygen radicals

There is evidence that RF-EMF may stimulate the formation of reactive oxygen species in exposed cells *in vivo* [35–37] and *in vitro* [38–41]. Free oxygen radicals may form base adducts in DNA, the most important lesion being 8-OHdG, and oxidize also other cellular components, such as lipids leaving behind reactive species, that in turn can couple to DNA bases [42]. The first step in the generation of ROS by microwaves is mediated in the plasma membrane by NADH oxidase [43]. Subsequently ROS activates matrix metalloproteases (MMP), thereby initiating intracellular signalling cascades. It is interesting to note that these processes start within 5 min of radiation and at a very low field intensity of 0.005 W/cm<sup>2</sup>. Moreover, higher effects have been obtained by intermittent radiation, when cells were left unirradiated for 10 min. This is in agreement with *in vitro* genotoxicity studies using the comet assay [23,33].

### 6.3. Alteration of DNA-repair processes

A considerable proportion of studies have investigated the consequences of a combined exposure to RF-EMF and various chemical or physical mutagens. 8/12 studies using human blood lymphocytes have demonstrated that RF-EMF enhanced the genotoxic action of other agents, preferentially of UV, MMC, or 4-NQ10 (an UV-mimetic agent). Since in all these experiments microwave exposure failed to induce detectable genotoxic effect by itself, an interference with DNA-repair mechanisms has been postulated, however, there is no direct experimental proof yet. An alteration of recombinational repair has also been proposed by Sykes et al. [22] as an explanation of the reduced rate of inversions in lacZ-transgenic mice after RF-EMF treatment.

An influence of microwave exposure on DNA-repair processes has long been proposed for power frequency electromagnetic fields [35]. A recent epidemiological investigation into the frequency of polymorphisms of DNA-repair genes in children with acute leukemia living in the vicinity of power line transformers [44] emphasizes the significance of DNA-repair impairment for an EMF related increase of this malignancy. There was a significant gene–environment interaction (COR = 4.31) between the electromagnetic field intensities and a less active genetic variant of XRCC1, a crucial enzyme in base excision repair.

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# Increased blood–brain barrier permeability in mammalian brain 7 days after exposure to the radiation from a GSM-900 mobile phone

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## Abstract

Microwaves were for the first time produced by humans in 1886 when radio waves were broadcasted and received. Until then microwaves had only existed as a part of the cosmic background radiation since the birth of universe. By the following utilization of microwaves in telegraph communication, radars, television and above all, in the modern mobile phone technology, mankind is today exposed to microwaves at a level up to  $10^{20}$  times the original background radiation since the birth of universe.

Our group has earlier shown that the electromagnetic radiation emitted by mobile phones alters the permeability of the blood–brain barrier (BBB), resulting in albumin extravasation immediately and 14 days after 2 h of exposure.

In the background section of this report, we present a thorough review of the literature on the demonstrated effects (or lack of effects) of microwave exposure upon the BBB.

Furthermore, we have continued our own studies by investigating the effects of GSM mobile phone radiation upon the blood–brain barrier permeability of rats 7 days after one occasion of 2 h of exposure. Forty-eight rats were exposed in TEM-cells for 2 h at non-thermal specific absorption rates (SARs) of 0 mW/kg, 0.12 mW/kg, 1.2 mW/kg, 12 mW/kg and 120 mW/kg. Albumin extravasation over the BBB, neuronal albumin uptake and neuronal damage were assessed.

Albumin extravasation was enhanced in the mobile phone exposed rats as compared to sham controls after this 7-day recovery period (Fisher's exact probability test,  $p = 0.04$  and Kruskal–Wallis,  $p = 0.012$ ), at the SAR-value of 12 mW/kg (Mann–Whitney,  $p = 0.007$ ) and with a trend of increased albumin extravasation also at the SAR-values of 0.12 mW/kg and 120 mW/kg. There was a low, but significant correlation between the exposure level (SAR-value) and occurrence of focal albumin extravasation ( $r_s = 0.33$ ;  $p = 0.04$ ).

The present findings are in agreement with our earlier studies where we have seen increased BBB permeability immediately and 14 days after exposure. We here discuss the present findings as well as the previous results of altered BBB permeability from our and other laboratories. © 2009 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** Albumin; Blood–brain barrier; Mobile phone; Rat

## 1. Introduction: radiofrequency radiation and the blood–brain barrier

Today about half of the world's population owns the microwave-producing mobile phones. An even larger number is exposed to the radiation emitted from these devices through "passive mobile phoning" [1]. Life-long exposure to the microwaves (MWs) from mobile phones, with start already at a young age, is becoming increasingly common

**Abbreviations:** BBB, blood–brain barrier; CNS, central nervous system; CW, continuous wave; EMF, electromagnetic field; GSM, global system for mobile communication; ICNIRP, International Commission of Non-ionizing Radiation Protection; MRI, magnetic resonance imaging; RF, radio frequency; SAR, specific absorption rate; TEM-cell, transverse electromagnetic transmission line chamber.

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among the new generations of mobile phone users. The question is: to what extent are living organisms affected by these radio frequency (RF) fields?

The mobile phones are held in close proximity to the head, or within a metre of the head when hands-free sets are used. The emitted microwaves have been shown to have many effects upon the mammalian brain; e.g. alterations of cognitive functions [2,3], changes of neurotransmitter levels such as decrease of cholinergic activity [4], gene expression alterations in cerebellum [5], cortex and hippocampus [6], and impact upon the brain EEG activity [7]. Also, the human brain EEG beta rhythms energies were increased by exposure to 450 MHz MWs modulated at different low frequencies [8]. Recent epidemiological studies also indicate that long-term exposure increases the risk of not only for benign vestibular schwannoma (previously named acoustic neurinoma) [9], but also malignant glioblastoma multiforme [10] for mobile phone use longer than 10 years and with cumulative exposure from mobile phones exceeding 2000 h.

It has been shown that electromagnetic fields (EMFs) increase the permeability of the blood–brain barrier (BBB) (for reference see [11]). The BBB is a hydrophobic barrier, formed by vascular endothelial cells of the capillaries in the brain, with tight junctions between these endothelial cells. It protects the mammalian brain from potentially harmful compounds in the blood. Also, perivascular structures such as astrocytes and pericytes as well as a bi-layered basal membrane help maintaining the BBB.

The current recommendations for limits of exposure to the general public for EMF radiation [12] are set in order to avoid thermal effects upon the brain parenchyma.

In our previous studies we have seen that non-thermal RF fields cause significantly increased leakage of the rats' own albumin through the BBB of exposed rats sacrificed immediately after the exposure, as compared to sham exposed control animals [11,13–18]. Two hours of exposure to the radiation from a global system for mobile communications (GSM) phone at 915 MHz, at non-thermal specific absorption rates (SAR) values of 0.12 mW/kg, 12 mW/kg and 120 mW/kg, gives rise to focal albumin extravasation and albumin uptake into neurons also 14 days after exposure, but not after 28 days [19]. Significant neuronal damage is present 28 days [19] and 50 days after exposure [20], but not after 14 days [19]. Also, in experiments from other laboratories, BBB permeability is increased in connection to mobile phone exposure [21–23] and other kinds of EMF such as magnetic resonance imaging (MRI) exposure [24–26]. In other studies, no such BBB alterations have been demonstrated in connection to mobile phone exposure [27–29] or other kinds of EMF exposure [30,31].

### 1.1. The blood–brain barrier

An intact BBB is necessary for the protection of the mammalian brain from potentially harmful substances circulating in the blood. In the normal brain, the passage of compounds over the BBB is highly restricted and homeostasis within

the sensitive environment of the brain parenchyma can be maintained.

The BBB is formed by the vascular endothelial cells of the capillaries of the brain and the glial cells wrapped around them. The tight junctions, that seal the endothelial cells together, limit paracellular leakage of molecules. A bi-layered basal membrane supports the abluminal side of the endothelial cells. The glial astrocytes, surrounding the surface of the basal membrane cells, are important for the maintenance, functional regulation and repair of the BBB. The protrusions of the astrocytes, called end feet, cover the basal membrane on the outer endothelial surface and thus form a second barrier to hydrophilic molecules and connect the endothelium to the neurons. Twenty-five per cent of the abluminal membrane of the capillary surface is covered by pericytes [32], which are a type of macrophages. Seemingly, they are in the position to significantly contribute to the central nervous system (CNS) immune mechanisms [33].

The physiological properties of the CNS microvasculature are different from those of peripheral organs. The numbers of pinocytotic vesicles for nutrient transport through the endothelial cytoplasm are low and there are no fenestrations. Also, there is a fivefold higher number of mitochondria in the BBB endothelial cells as compared to muscular endothelial cells [34].

In a functioning BBB, the membrane properties control the bidirectional exchange between the general circulation and the CNS. Water, most lipid-soluble molecules, oxygen and carbon dioxide can diffuse from the blood to the nerve cells. The barrier is slightly permeable to ions such as sodium, potassium and chloride, but large molecules, such as proteins and most water-soluble chemicals only pass poorly. However, when this barrier is damaged, in conditions such as tumours, infarcts or infections, also the normally excluded molecules can pass through, possibly bringing toxic molecules out into the brain tissue. The selective permeability is disrupted temporally in cases of epileptic seizures [35,36] and severe hypertension [37]. The result of this can be cerebral oedema, increased intracranial pressure and irreversible brain damage. Also, toxic substances from the blood circulation now reach out to the neurons. Even transient openings of the BBB can lead to permanent tissue damage [37].

### 1.2. The earliest studies on the effects of microwave exposure

The first studies on the MW effects upon the BBB were reported in the 1970s, when the radiation from radars and MW ovens were considered to be possible health threats. Increased leakage of fluorescein after 30 min of pulsed and CW exposure [38] and passage of  $^{14}\text{C}$ -mannitol, inulin and dextran at very low energy levels [39] were reported. The permeation of mannitol was found to be a definite function of exposure parameters such as power density, pulse width, and the number of pulses per second. Also, the BBB permeability depended on the time between the EMF exposure and the

sacrifice of the animals, with more pronounced effects seen in the animals sacrificed earlier after the EMF exposure. In attempts to replicate the findings of Oscar and Hawkins [39], however, these results were not found [40,41]. Similar lack of MW induced BBB effects, was reported by Ward et al. [42] after exposure of rats to CWs at 2450 MHz; Ward and Ali [43] after exposure at 1.7 GHz; and Gruenau et al. [44] after exposure to pulsed or CW waves at 1.8 GHz (including totally 31 rats). On the other hand, Albert and Kerns [45] observed EMF-induced BBB permeability after exposure at 2450 MHz CWs, with an increase in the number of pinocytotic vesicles among the irradiated animals, but after a recovery time of 1–2 h, the permeation was hardly detectable anymore. For details concerning the EMF exposure parameters in these studies, see [11].

### 1.3. MRI exposure

MRI entails a concurrent exposure to a high-intensity static field, a RF field and a time-varying magnetic field. In connection to the introduction of the MRI technique, the effects of exposure to these kinds of fields upon the BBB permeability were investigated.

As mentioned above, Shivers et al. [24] observed that the EMF exposure of the type emitted during a MRI procedure resulted in a temporarily increased BBB permeability in the brains of rats. Through transendothelial channels, a vesicle-mediated transport of horseradish peroxidase (HRP) took place. Replications of the initial findings by Shivers et al. [24] were made by Garber et al. [46], whereas Adzamlı et al. [30] and Preston et al. [31] could not repeat the findings.

After some years, quantitative support of the findings by Shivers et al. [24] was presented by the same group [25,26]. In rats exposed to the MRI, the BBB permeability to DTPA (diethylenetriaminepentaacetic acid) increased. A suggested mechanism explaining the increased permeability was a stimulation of endocytosis, made possible through the time-varying magnetic fields.

Also our studies supported the findings of the Shiver–Prato group; seeing that BBB permeability to albumin was increased after exposure to MRI radiation [13]. The most significant effect was observed after exposure to the RF part of the MRI.

### 1.4. Studies on mobile phone exposure

The mobile phone induced effects upon the BBB permeability is a topic of importance for the whole society today. We have previously found an increased BBB permeability immediately after 2 h of mobile phone exposure [14], and also after 14 days [19] and 50 days [20].

Repetitions of our findings of increased BBB permeability after mobile phone exposure have been made [47,21,22]. Four hours of GSM-900 MHz exposure at brain power densities ranging from 0.3 to 7.5 W/kg resulted in significantly increased albumin extravasation both at the SAR-value of

7.5 W/kg, which is a thermal effect, but also at 0.3 W/kg and 1.3 W/kg [47] (statistical evaluations discussed by Salford et al. [1]). Albumin extravasation was also seen in rats exposed for 2 h to GSM-900 MHz at non-thermal SAR-values of 0.12, 0.5 and 2 W/kg using fluorescein-labelled proteins [21,22]. At SAR of 2 W/kg a marked BBB permeabilization was observed, but also at the lower SAR-value of 0.5 W/kg, permeabilization was present around intracranial blood vessels. However, the extravasation at 0.5 W/kg was seen at a lesser extent as compared to that seen at 2 W/kg. Subgroups of the rats included in these studies were sympathectomised, which means that they were in a chronic inflammation-prone state with increased BBB opening due to changes in the structures of the blood vessels. Interestingly, the sympathectomised rats exposed to GSM radiation had a remarkable increase of the BBB leakage as compared to their sympathectomised sham controls. From these findings it seems likely that an already disrupted BBB is more sensitive to the RF fields than an intact BBB.

In another study, the uptake of rhodamine–ferritin complex through the BBB was investigated [23]. In this study, increased BBB permeability was clearly seen at exposure levels of 2 W/kg and durations of 30–120 min. When the rats were pre-treated with colchicine, the EMF-induced rhodamine–ferritin uptake was however blocked. Colchicine is well-known for its inhibition of microtubular function. It was concluded that the microtubules seemed to play an important role for the BBB opening.

Lack of EMF-induced BBB alterations has also been reported [27–29,48]. In a small study including only 12 EMF exposed animals, no albumin extravasation was seen, neither after 2 nor 4 weeks of 1 h of daily exposure (average whole-body exposure at 0.25 W/kg) [27]. In a study including 40 animals, Kuribayashi et al. [28] concluded no BBB alterations was seen after 90 min of daily EMF exposure for 1–2 weeks at SAR-values of 2 or 6 W/kg. Finnie et al. [29] exposed mice for 1 h daily. However, only the SAR-value of 4 W/kg, which is above the ICNIRP limit [12], was included. In a further study by Finnie et al. [48] 207 mice were exposed for 104 weeks at SAR-values of 0.25–4 W/kg, however without any observable effects upon the BBB permeability. The same group also reported that the immature BBB was insensitive to mobile phone exposure, seen after GSM-900 radiation exposure of pregnant mice from day 1 to day 19 of gestation (SAR of 4 W/kg, exposure for 60 min daily). No increased albumin extravasation was seen in the new-born mice immediately after parturition [49] and the same lack of GSM-900 radiation effects upon the BBB permeability was reported for young rats by Kumlin et al. [50], however, in this case only 12 out of totally 48 exposed rats were analyzed histopathologically. The remaining rats were subject to memory tests, where an improved learning and memory was seen in the EMF exposed rats as compared to the sham controls. Notably, in all these studies, the SAR-values for exposure are relatively high; never including the low SAR-values below 200 mW/kg.

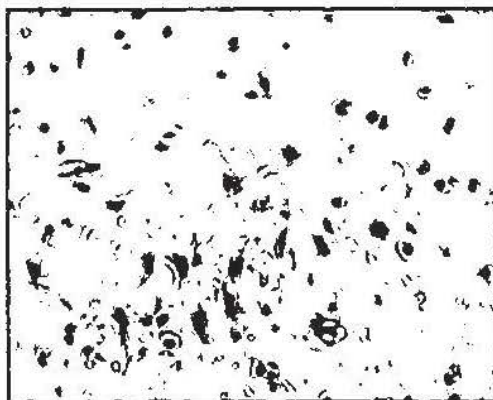


Fig. 1. Albumin neuronal uptake and early neuropathy in the hippocampal pyramidal cell row among normal neurons. Albumin: cresyl violet,  $\times 20$ .

In more recent years, *in vitro* models have been increasingly applied to investigate the BBB; in one of these, it was shown that EMFs at 1.8 GHz increase the permeability to sucrose [51]. After modifications of the BBB model to one with higher tightness, however, the same group could not replicate their initial findings [52]. With application of the EMF of the kind emitted from 3G mobile phones, the same group further concluded that their *in vitro* BBB model also did not alter its tightness or transport behaviour in connection to this type of exposure [53].

#### 1.5. Neuronal damage in connection to mobile phone exposure

In our previous studies of animals surviving a longer period after the exposure, we have evaluated the occurrence of neuronal damage extensively [19,20]. This neuronal damage is seen as condensed dark neurons. Dark neurons have been proposed to have three main characteristics [54]: (i) irregular cellular outlines, (ii) increased chromatin density in the nucleus and cytoplasm and (iii) intensely and homogeneously stained nucleus. Twenty-eight days after 2 h of mobile phone exposure, the neuronal damage was significantly increased in the exposed rats as compared to the sham exposed controls [19]. Also 50 days after the same kind of mobile phone exposure, there was an increased occurrence of neuronal damage [20].

In our studies, normal neurons have been shown to have increased uptake of albumin [19] (Fig. 1). Also, in dark neurons this neuronal albumin uptake can be seen (Fig. 2). In our previous studies, damaged neurons were seen in all locations, intermingled with normal neurons especially in the cortex, hippocampus and basal ganglia. The damaged neurons were often shrunken and dark staining, homogenized with loss of discernable internal cell structures (Fig. 3). Some damaged neurons showed microvacuoles in the cytoplasm (Fig. 4). These vacuoles are a sign of severe neuronopathy, indicating an active pathological process. There was no evidence of haemorrhages or glial reaction.

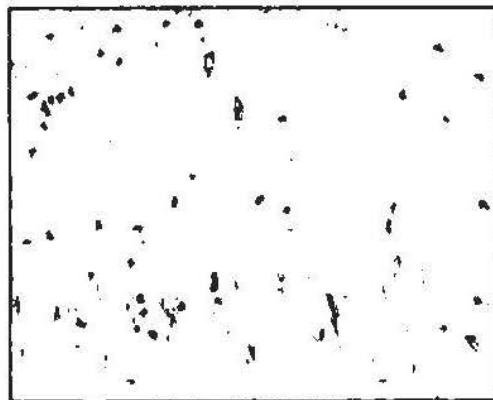


Fig. 2. Shrunken homogenized dark neurons with brownish discoloration due to uptake of albumin, interspersed among normal neurons in the hippocampal pyramidal cell row. Albumin: cresyl violet,  $\times 20$ . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)



Fig. 3. Two dark neurons in the hippocampal pyramidal cell row. Albumin: cresyl violet,  $\times 20$ .

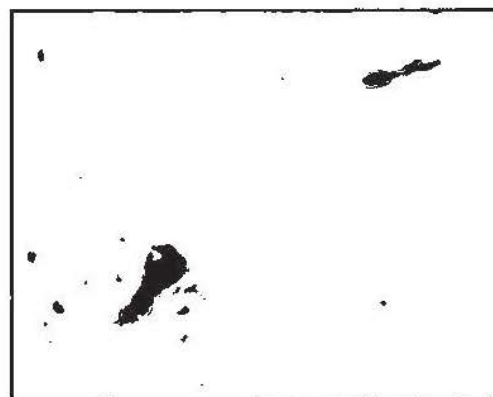


Fig. 4. Dark neuron in the hippocampal pyramidal cell row, with homogenized nucleus and cytoplasm with a vacuole. Higher magnification of part of the figure. Albumin: cresyl violet,  $\times 40$ .

Dark neurons are reported in clinical and experimental neuropathology from living tissues, but not in autopsy material unless the post-mortem period is short. This could indicate that the formation of dark neurons is an active process that requires living neurons and that these cells must be reasonably intact [55]. This could be in accordance with our findings from the 50-days survival animals, where apoptosis could not be detected in any of the RF EMF exposed brains with application of Caspase-3 [56].

Dark neurons occur not only after GSM exposure [19,20] but also in connection to experimental ischemia [57], hypoglycemia [58] and epilepsy [59]. Possibly, dark neurons could be artefacts, having a pressure-derived mechanical origin, as has been shown for cortical biopsies (this is less likely considering the atraumatic method of dissection used here including fixation before handling and in view of the deep location of the dark neurons). However, dark neurons also appear as a result of other, and not fully clarified, mechanisms, as seen in the case of GSM exposure, ischemia, hypoglycemia and epilepsy. A pharmacologic origin, such as depolarization related to tissue glutamate release in injury, could explain the pathogenetic mechanism for dark neurons in these cases, rather than the pressure-derived mechanical origin. Indeed, the formation of dark neurons can be prevented using pharmacologic forms of glutamate antagonism [55]. In the case of our studies, our technique for the resection of the rat brains is chosen to avoid mechanical pressure.

Findings of dark neurons in connection to mobile phone exposure have been reported by Ihan et al. [60] (GSM exposure of rats for 7 days, 1 h daily). Also, an increase of oxidative damage was seen in the exposed rats as a significant increase in malondialdehyde (MDA) (an index for lipid peroxidation), nitric oxide (NO) levels, brain xanthine oxidase (XO) and adenosine deaminase (ADA) activities, as compared to the controls. With treatment of the anti-oxidant *Ginkgo biloba*, the EMF induced increments of XO, ADA, MDA and NO were prevented. The anti-oxidant activity of *G. biloba* is attributed to its flavinoid glycosides, which are the active compounds in the leaves. The action of these flavinoids is to destroy free radicals, such as NO and lipid peroxide radicals. Also the formation of dark neurons was reported to be prevented when the rats had been treated with *G. biloba*. Other attempts to repeat our findings of dark neurons after mobile phone exposure have been performed in a collaborative effort with Bernard Veyret's group in Bordeaux [61]. In this study, the situation 14 days and 50 days after 2 h of GSM-900 radiation exposure at average brain SAR-values of 0.14 W/kg and 2.0 W/kg was evaluated. No increased amount of dark neurons was reported.

It has been suggested that BBB leakage is the major reason for nerve cell injury, such as dark neurons in stroke-prone spontaneously hypertensive rats [62]. Albumin leaks into the brain and neuronal degeneration is seen in areas with BBB disruption in several circumstances: after intracarotid infusion of hyperosmolar solutions in rats [63]; in the stroke prone hypertensive rat [65]; in acute hypertension by aor-

tic compression in rats [37]. The linkage between albumin extravasation over the BBB and neural damage might be a potentiating effect of albumin upon the glutamate-mediated neurotoxicity [64]. Indeed, both albumin- and glutamate-induced lesions have the same histopathological appearance with invasion of macrophages and absence of neuronal cell bodies and axons in the lesion areas [65]. The glutamate itself can also increase the BBB opening [66], leading to further albumin extravasation out into the brain parenchyma. From our previous findings of albumin extravasation 14 days after exposure [19] and dark neurons not until after 28 days [19] and 50 days [20], it could be hypothesized that albumin extravasation into the brain parenchyma, is the first observable effect of the mobile phone exposure. The albumin leakage precedes and possibly could be the cause of, the damage to the neurons seen as the dark neurons later on. In this connection, the findings of [37] that transient openings of the BBB can result in permanent tissue damage, can also be mentioned. Hypertensive opening of the BBB resulted in albumin extravasation after 2 h, but the effects remained, although to a lesser extent, also after 7 days. Many neurons with cytoplasmatic immunoreactivity for albumin appeared shrunken. Seven days after the BBB opening, there was a neuronal loss in these areas and a vigorous glial reaction, indicating that some neurons were irreversibly damaged [37].

## 2. Aims of the present study

In the present study we have continued to investigate the effects of EMFs upon the rat brain, now with focus on what happens 7 days after GSM exposure at 915 MHz for 2 h at non-thermal energy levels of 0.12 mW/kg, 1.2 mW/kg, 12 mW/kg and 120 mW/kg. The main questions to be answered were: whether the same increase of the BBB permeability is seen 7 days after exposure as that showed previously immediately after exposure and after 14 days, and whether different exposure levels result in a different response.

In order to compare to our previous findings, we have used the same exposure system, GSM signal, animal model and histopathological methods as in our previous studies.

## 3. Materials and methods

### 3.1. GSM exposure

The animals were exposed to RF EMFs in the same transverse electromagnetic transmission line cell (TEM-cells) as previously described and used by [1,2,5,13–19]. The TEM-cells were designed by dimensional scaling from previously constructed cells at the National Bureau of Standards [67]. TEM-cells are known to generate uniform EMFs for standard measurements.

A genuine GSM mobile phone, operating at the 900 MHz frequency band, with programmable power output, was con-

nected via a coaxial cable to the TEM-cells. Through a power splitter, the power was divided into equal parts fed into the four TEM-cells used (TEM-cell A, B, C and D). No voice modulation was applied. Each of the four TEM-cells is connected to a 50  $\Omega$  dummy load, into which the output is terminated. By using these TEM-cells, the pulse modulated exposure fields can be accurately generated without the distortion that is typically introduced when conventional antennas are used to establish impulse test fields. Thus, a relatively homogeneous exposure of the animals is allowed [68].

The TEM-cell is enclosed in a wooden box (inner dimensions of 15 cm  $\times$  15 cm  $\times$  15 cm) that supports the outer conductor, made of brass net, and central conducting plate. The central plate separates the top and bottom of the outer conductor symmetrically. Eighteen holes (diameter 18 mm) in the sidewalls and top of the wooden box make ventilation possible. Air is circulated through the holes of the TEM-cells using four fans, each placed next to the outer walls of its respective TEM-cell. The holes are also used for examination of the interior during exposure. For a further description of the TEM-cell, see [68].

The rats were placed in plastic trays (14 cm  $\times$  14 cm  $\times$  7 cm) to avoid contact with the central plate and outer conductor. The bottom of the tray was covered with absorbing paper to collect urine and faeces. Each TEM-cell contained two plastic trays, one above and one below the centre septum. Thus two rats could be kept in each TEM-cell simultaneously. All the animals could move and turn around within the TEM-cells.

For the actual experimental situation with one rat in each compartment of the TEM-cell, the conversion factor  $K$  for SAR per unit of input power could be fitted to the data as

$$K = (1.39 \pm 0.17) - (0.85 \pm 0.22)w \quad (1)$$

with  $w$  the sum of weights in kilograms of the 2 rats in the cell and the variance given as SEM. For a more detailed description, see [2].

Whole-body SAR and brain SAR vary with orientation. In our present set-up, the average of SAR for the brain grey matter was 1.06 times the average whole-body SAR, with a standard deviation of 56% around the average value for the different orientations, as estimated by us previously [19].

### 3.2. Animals

All animal procedures were performed according to the practices of the Swedish Board of Animal Research and were approved by the Animal Ethics Committee, Lund-Malmö.

Forty-eight inbred male and female Fischer 344 rats (the rats were supplied by Scanbur AB, Stockholm, Sweden) were 2–3 months of age at the initiation of the EMF exposure. Male and female rats weighed  $225 \text{ g} \pm 56 \text{ g}$  (standard deviation) and  $233 \text{ g} \pm 60 \text{ g}$  (standard deviation) respectively.

The rats were housed in rat hutches, two in each cage, under standard conditions of 22°C room temperature, artificial daylight illumination and rodent chow and tap water *ad libitum*.

The 48 rats were divided into four exposure groups, each group consisting of 8 rats, and one sham exposed group with 16 animals.

The peak power output from the GSM mobile phone fed into the TEM-cells was 1 mW, 10 mW, 100 mW and 1000 mW per cell respectively for a period of 2 h. This resulted in average whole-body SAR of 0.12 mW/kg, 1.2 mW/kg, 12 mW/kg and 120 mW/kg for the four different exposure groups.

All animals were kept in the animal facilities for a recovery period of 7 days after exposure. At the end of this period they were anaesthetized and sacrificed by perfusion-fixation with 4% formaldehyde.

### 3.3. Histopathology and methods

The brains were fixed *in situ* through saline perfusion through the ascending aorta for 3 min followed by 4% formaldehyde for 10 min and immersion in 4% formaldehyde for 24 h. They were then removed from the skulls by a non-traumatic technique (resection of bone structures at the skull base, followed by a midline incision from the foramen magnum to the nose) and immersion fixed in 4% formaldehyde for more than 24 h. Whole coronal sections of the brains were dehydrated and embedded in paraffin, sectioned at 5  $\mu\text{m}$  with a microtome and stained for RNA/DNA with cresyl violet to visualize damaged neurons. Albumin was demonstrated with the IgG fraction of rabbit anti-rat albumin (Dakopatts, Helsingborg, Sweden) diluted 1:8,000. This reveals albumin as brownish spotty or more diffuse discolorations. Biotinylated swine anti-rabbit IgG was used as a secondary antibody. Then avidin, peroxidase conjugated, was coupled to the biotin and visualized with DAB (diaminobenzidine).

All brains were examined histopathologically by our neuropathologist (A.B.). All microscopic analyses were performed blind to the test situation.

Regarding albumin extravasation, the number of immunopositive extravasates (foci) were recorded under a microscope. None or occasional minor leakage was rated as normal, whereas one larger or several leakages were regarded as pathological. Immunopositive sites were, however, disregarded when localized in the hypothalamus, above the median eminence and laterally including the lateral hypothalamic nuclei, in the immediate vicinity of the third ventricle and just beneath the pial membrane. These structures are well known for their insufficient BBB. Also the presence and distribution of albumin uptake into neurons was judged semi-quantitatively.

Regarding neuronal damage, this were judged semi-quantitatively as no or occasional (score 0), moderate (score 1) or abundant occurrence (score 2) of dark neurons.

### 3.4. Statistics

As an initial discriminative test, the occurrence of an effect of exposure (score 1 or higher for albumin foci; score 0.5 or higher for neuronal albumin uptake and dark neurons) was tested against sham exposed controls using Fisher's exact probability test.

The Kruskal–Wallis one-way analysis of variance by ranks was used for a simultaneous statistical test of the score distributions for the five conditions of sham or EMF exposure. When the null hypothesis could be rejected, the non-parametric Mann–Whitney *U*-test for independent samples was used to compare each of the groups of GSM exposed and sham exposed animals.

The occurrence of covariates such as gender, the position of the rat in the TEM-cell (upper/lower compartment) and the TEM-cell used (TEM-cell A, B, C or D) was evaluated using linear regression analysis.

Spearman's non-parametric correlation analysis was used for evaluation of correlation between exposure level, albumin foci, neuronal albumin and dark neurons.

## 4. Results

In exposed animals there were albumin positive foci around capillaries in the white and grey matter (Fig. 5). The albumin had diffused into the neuropil between the cell bodies, surrounding the neurons, which either contained no albumin or contained albumin in some foci. Scattered neurons were albumin positive. Regarding the dark neurons, cresyl violet staining showed that these were scattered and sometimes grouped within the brain parenchyma.

The occurrence of albumin outside brain vessels was characterized as albumin foci around vessels. After the 7 days recovery time, albumin foci were found significantly more often among exposed rats (25%) than among sham exposed

rats (0%) (Fisher's exact probability test,  $p = 0.04$ ). There was a low, but significant correlation between the exposure level (SAR-value) and the occurrence of albumin foci (Spearman analysis,  $r_s = 0.33$ ;  $p = 0.04$ ). Taking the level of exposure and quantification of neuropathological effects into account it could be concluded from a simultaneous non-parametric comparison of all 5 exposure levels with the Kruskal–Wallis test, that the distribution of albumin foci differed significantly (Kruskal–Wallis,  $p = 0.012$ ).

Pair-wise comparisons between the different exposure levels and sham exposed animals revealed statistically significant differences for SAR of 12 mW/kg (Mann–Whitney,  $p = 0.007$ ), whereas a trend of increased albumin extravasation could be seen for 0.12 mW/kg (Mann–Whitney,  $p = 0.1$ ) and 120 mW/kg (Mann–Whitney,  $p = 0.1$ ).

Also, the occurrence of neuronal albumin was evaluated. A simultaneous analysis for all exposure levels revealed a significant difference between the five groups (Kruskal–Wallis,  $p = 0.03$ , however Fisher's exact probability,  $p = \text{ns}$ ). A pair-wise comparison revealed that albumin uptake occurred more frequently at 1.2 mW/kg as compared to sham exposed (Mann–Whitney,  $p = 0.02$ ). No difference was found for the occurrence of neuronal damage (Kruskal–Wallis,  $p = \text{ns}$ ; Fisher's exact probability test,  $p = \text{ns}$ ).

Linear regression analysis did not reveal any influence of gender, position of the animals in the TEM-cell (upper/lower compartment) or the TEM-cell used (TEM-cell A, B, C or D) on the frequency of albumin foci, neuronal albumin or occurrence of dark neurons.

## 5. Discussion

The present study provides evidence that GSM exposure results in disruption of the BBB permeability, with remaining, observable effects 7 days after the exposure occasion. Only non-thermal SAR-levels, below the limits of allowed exposure for humans [12] are considered. This finding of increased albumin extravasation after 7 days (Kruskal–Wallis,  $p = 0.012$  with all animals included in the analysis, which is also in agreement with the Fisher's exact probability test,  $p = 0.04$ ) is in line with our earlier findings of albumin leakage both immediately following 2 h of GSM exposure [16] and 14 days [19] after 2 h of GSM exposure. Also, the increased occurrence of neuronal albumin 7 days after the exposure is in line with the findings 14 days after exposure [19].

In our previous study, where the animals have been sacrificed immediately after the EMF exposure, we have seen albumin extravasation only at the most in 50% of the identically exposed animals, although all animals are inbred Fischer 344 rats [16]. Among the rats exposed to the pulse modulated EMFs at 915 MHz, 35% showed albumin extravasation. Also in the sham exposed animals, albumin leakage was present (in 17% of the animals). When the animals have survived 7 days after the EMF exposure, albumin extravasation is seen in a lesser proportion (25% of the exposed



Fig. 5. Focal leakage of albumin shown in brown in the cortex. Albumin: cresyl violet,  $\times 10$ . GSM-900 EMF exposure at 12 mW/kg. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

animals) and in none of the sham controls. This could be due to a rapid diffusion of extravasated albumin down to, and beyond concentrations possible to demonstrate immunohistochemically. Numerous routes of clearance of extravasated molecules out from the brain tissue are present in the living brain and compounds can also become sequestered intracellularly, become protein bound or metabolized. After 14 days, albumin extravasation is seen in a somewhat larger proportion of the EMF exposed animals (29% of the exposed animals) and none of the sham controls. Thus, a secondary BBB opening might have started at some time point after the initial opening, leading to a vicious circle of albumin leakage.

The mechanism for the passage of albumin over the BBB is not clear. Extravasation might occur through paracellular routes, including alterations of tight junctions between the vascular endothelial cells, or transcellular routes with induction of pinocytosis or transcytosis, formation of transendothelial channels or disruption of the endothelial cell membrane. In connection to EMF exposure, amplified vesicle-mediated transport across the microvessel endothelium occurs, including also transendothelial channels, but no passage through disrupted inter-endothelial tight junctions [24].

One remarkable observation is that exposure at very low whole-body average power densities gives rise to a pronounced albumin leakage. In the present study, significant effects could be seen already at 12 mW/kg, although the different animal groups included a relatively small number of animals. Most certainly, the trends seen for exposure levels of 0.12 mW/kg and 120 mW/kg would have reached statistical significance if more animals had been included in the different exposure groups.

The phenomenon with increased BBB permeability already at very low energy levels might represent a U-curve response. In our other studies, we have seen that the rats in several of the groups with different SAR-levels of EMF exposure have a significant BBB opening [16,19]. The U-response curve occurs also in connection with other kinds of MW exposure, where cerebral vessel permeability after an initial rise decreased with increasing MW power [39].

Further investigation of BBB permeability in connection to EMF exposure is important not only in order to reduce the potentially harmful effects, but also to use possible beneficial effects [69]. The transport of drugs over the BBB might be regulated, so that targets within the brain can be reached. For example, steering of BBB passage of the antiretroviral agent saquinavir has been accomplished in an *in vitro* model of the human BBB, where a frequency of 915 MHz generated the highest BBB permeability [69]. This could be extremely important in order to reduce the HIV replication in the brain of HIV-infected individuals.

## 6. In conclusion

The time between EMF exposure and sacrifice of the animals is of great importance for the detection of albumin foci.

Seven days after 2 h of GSM mobile phone exposure, there is still an increased permeability of the BBB of exposed rats. This is in concordance with earlier findings of albumin extravasation out into the brain parenchyma immediately and 14 days after 2 h of mobile phone exposure.

## 7. General conclusion

Taken together, it can be concluded that in a number of studies MW effects upon the BBB permeability have been observed. Increased permeability can be seen both immediately after exposure, but also 7 days after the exposure, as reported in this primary report, and after 14 days. It seems that the effects of the MW radiation might result in persistent changes, such as those seen in our own studies with neuronal damage both 28 and 50 days after 2 h of mobile phone exposure. In a future perspective, with increasing number of active mobile phone users, passive mobile phoning, radiation emitted from base stations and also MWs emitted from other communication sources, effects of low non-thermal levels of exposure must be considered further. The effects seen in the rat studies give some clues about what might possibly happen in the human brain, with a BBB very similar to that of rats. While awaiting latency periods long enough for adequate epidemiological interpretations, further studies on both animals and cells are of utmost importance.

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## Epidemiological evidence for an association between use of wireless phones and tumor diseases

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### Abstract

During recent years there has been increasing public concern on potential cancer risks from microwave emissions from wireless phones. We evaluated the scientific evidence for long-term mobile phone use and the association with certain tumors in case-control studies, mostly from the Hardell group in Sweden and the Interphone study group. Regarding brain tumors the meta-analysis yielded for glioma odds ratio (OR) = 1.0, 95% confidence interval (CI) = 0.9–1.1. OR increased to 1.3, 95% CI = 1.1–1.6 with 10 year latency period, with highest risk for ipsilateral exposure (same side as the tumor localisation), OR = 1.9, 95% CI = 1.4–2.4, lower for contralateral exposure (opposite side) OR = 1.2, 95% CI = 0.9–1.7. Regarding acoustic neuroma OR = 1.0, 95% CI = 0.8–1.1 was calculated increasing to OR = 1.3, 95% CI = 0.97–1.9 with 10 year latency period. For ipsilateral exposure OR = 1.6, 95% CI = 1.1–2.4, and for contralateral exposure OR = 1.2, 95% CI = 0.8–1.9 were found. Regarding meningioma no consistent pattern of an increased risk was found. Concerning age, highest risk was found in the age group <20 years at time of first use of wireless phones in the studies from the Hardell group. For salivary gland tumors, non-Hodgkin lymphoma and testicular cancer no consistent pattern of an association with use of wireless phones was found. One study on uveal melanoma yielded for probable/certain mobile phone use OR = 4.2, 95% CI = 1.2–14.5. One study on intratemporal facial nerve tumor was not possible to evaluate due to methodological shortcomings. In summary our review yielded a consistent pattern of an increased risk for glioma and acoustic neuroma after >10 year mobile phone use. We conclude that current standard for exposure to microwaves during mobile phone use is not safe for long-term exposure and needs to be revised.

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**Keywords:** Brain tumors; Glioma; Acoustic neuroma; Meningioma; Cellular phones; Cordless phones

### 1. Introduction

During the last decade there has been a rapid development of wireless technology and along with that an increased use of wireless telephone communication in the world. Most persons use mobile phones and cordless phones. Additionally most populations are exposed to radiofrequency/microwave (RF) radiation emissions from wireless devices such as cellular antennas and towers, broadcast transmission towers, voice and data transmission for cell phones, pagers and personal digital assistants and other sources of RF radiation.

Concerns of health risks have been raised, primarily an increased risk for brain tumors, since the brain is the near field

target organ for microwave exposure during mobile phone calls. Especially the ipsilateral brain (same side as the mobile phone has been used) is exposed, whereas the contralateral side (opposite side to the mobile phone) is much less exposed [1]. Thus, for risk analysis it is of vital importance to have information on the localisation of the tumor in the brain and which side of the head that has been predominantly used during phone calls.

Since Sweden was one of the first countries in the world to adopt this wireless technology a brief history is given in the following. First, analogue phones (NMT; Nordic Mobile Telephone System) were introduced on the market in the early 1980s using both 450 and 900 Megahertz (MHz) carrier waves. NMT 450 was used in Sweden since 1981 but closed down in December 31, 2007, whereas NMT 900 operated during 1986–2000.

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Table 1

Odds ratios (ORs) and 95% confidence intervals (CIs) from 11 case-control studies on glioma including meta-analysis of the studies. Numbers of exposed cases and controls are given.

| Author, year of publication, country, reference number                 | No. of cases       | No. of controls    | OR  | 95% CI  |
|--|--------------------|--------------------|-----|---------|
| Inskip et al., 2001, USA [23]  | 201                | 358                | 1.0 | 0.7–1.4 |
| Auvinen et al., 2002, Finland [24]                                     | Not given          | Not given          | 1.5 | 1.0–2.4 |
| Lönn et al., 2005, Sweden [25] <sup>a</sup>                            | 214                | 399                | 0.8 | 0.6–1.0 |
| Christensen et al., 2005, low-grade glioma, Denmark [26] <sup>a</sup>  | 47                 | 90                 | 1.1 | 0.6–2.0 |
| Christensen et al., 2005, high-grade glioma, Denmark [26] <sup>a</sup> | 59                 | 155                | 0.6 | 0.4–0.9 |
| Hepworth et al., 2006, UK [27] <sup>a</sup>                            | 508                | 898                | 0.9 | 0.8–1.1 |
| Schüz et al., 2006, Germany [28]                                       | 138                | 283                | 1.0 | 0.7–1.3 |
| Hardell et al., 2006, Sweden [12], all glioma                          | 346                | 900                | 1.4 | 1.1–1.7 |
| Low-grade glioma   | 65                 | 900                | 1.4 | 0.9–2.3 |
| High-grade glioma  | 281                | 900                | 1.4 | 1.1–1.8 |
| Lahkola et al., 2006, Denmark, Norway, Finland, Sweden, UK [29]        | 867                | 1 853              | 0.8 | 0.7–0.9 |
| Hours et al., 2007, France [30]  | 59                 | 54                 | 1.2 | 0.7–2.1 |
| Klaeboe et al., 2007, Norway [31] <sup>a</sup>                         | 161                | 227                | 0.6 | 0.4–0.9 |
| Takebayashi et al., 2008, Japan [17]                                   | 56                 | 106                | 1.2 | 0.6–2.4 |
| Meta-analysis  | >1667 <sup>b</sup> | >3554 <sup>b</sup> | 1.0 | 0.9–1.1 |

<sup>a</sup> Not included in meta-analysis because already part of pooled data in Lahkola et al., 2006 [29].

<sup>b</sup> Total number could not be calculated since numbers were not presented in one publication [24].

The digital system (GSM; Global System for Mobile Communication) using dual band, 900 and 1800 MHz, started to operate in 1991 and now dominates the market. The third generation of mobile phones, 3G or UMTS (Universal Mobile Telecommunication System), using 1900 MHz RF broad band transmission has been introduced worldwide since a few years, in Sweden since 2003.

Desktop cordless phones have been used in Sweden since 1988, first analogue 800–900 MHz RF fields, but since early 1990s the digital 1900 MHz DECT (Digital Enhanced Cordless Telecommunications) system is used. In our studies on tumor risk associated with use of wireless phones, we have also assessed use of cordless phones. However, most other

research groups have not published such data at all, or only in a scanty way, so exposure to RF from DECT is not further discussed here. Instead the reader is referred to our previous publications on this issue [2–13].

The initial studies on brain tumor risk had too short latency periods to give a meaningful interpretation. However, during recent years studies have been published that enable evaluation of  $\geq 10$ -years latency period risk, although still mostly based on low numbers [14,15]. A  $\geq 10$ -years latency period seems to be a reasonable minimum period to indicate long-term carcinogenic risks from exposure to RF fields during use of mobile or cordless phones.

Table 2

Odds ratios (ORs) and 95% confidence intervals (CIs) from six case-control studies on glioma including meta-analysis of the studies using  $\geq 10$  year latency period. Numbers of exposed cases and controls are given.

| Study   | Total                 |      |         | Ipsilateral           |     |          | Contralateral         |     |         |
|---|-----------------------|------|---------|-----------------------|-----|----------|-----------------------|-----|---------|
|   | No. of cases/controls | OR   | 95% CI  | No. of cases/controls | OR  | 95% CI   | No. of cases/controls | OR  | 95% CI  |
| Lönn et al., 2005, Sweden, $\geq 10$ years [25] <sup>a</sup>                            | 25/38                 | 0.9  | 0.5–1.5 | 15/18                 | 1.6 | 0.8–3.4  | 11/25                 | 0.7 | 0.3–1.5 |
| Christensen et al., 2005, Denmark, low-grade glioma, $\geq 10$ years [26] <sup>a</sup>  | 6/9                   | 1.6  | 0.4–6.1 | –                     | –   | –        | –                     | –   | –       |
| Christensen et al., 2005, Denmark, high-grade glioma, $\geq 10$ years [26] <sup>a</sup> | 8/22                  | 0.5  | 0.2–1.3 | –                     | –   | –        | –                     | –   | –       |
| Hepworth et al., 2006, UK, $\geq 10$ years [27] <sup>a</sup>                            | 66/112                | 0.9  | 0.6–1.3 | Not given             | 1.6 | 0.9–2.8  | Not given             | 0.8 | 0.4–1.4 |
| Schüz et al., 2006, Germany, $\geq 10$ years [28]                                       | 12/11                 | 2.2  | 0.9–5.1 | –                     | –   | –        | –                     | –   | –       |
| Hardell et al., 2006, Sweden, $>10$ years [12], all glioma                              | 78/99                 | 2.7  | 1.8–3.9 | 41/28                 | 4.4 | 2.5–7.6  | 26/29                 | 2.8 | 1.5–5.1 |
| Low-grade glioma  | 7/99                  | 1.5  | 0.6–3.8 | 2/28                  | 1.2 | 0.3–5.8  | 4/29                  | 2.1 | 0.6–7.6 |
| High-grade glioma   | 71/99                 | 3.1  | 2.0–4.6 | 39/28                 | 5.4 | 3.0–9.6  | 22/29                 | 3.1 | 1.6–5.9 |
| Lahkola et al., 2006, Denmark, Norway, Finland, Sweden, UK, $\geq 10$ years [29]        | 143/220               | 0.95 | 0.7–1.2 | 77/117                | 1.4 | 1.01–1.9 | 67/121                | 1.0 | 0.7–1.4 |
| Meta-analysis   | 233/330               | 1.3  | 1.1–1.6 | 118/145               | 1.9 | 1.4–2.4  | 93/150                | 1.2 | 0.9–1.7 |

<sup>a</sup> Not included in meta-analysis because already part of pooled data in Lahkola et al., 2006 [29].

Table 3

Odds ratios (ORs) and 95% confidence intervals (CIs) from nine case-control studies on acoustic neuroma including meta-analysis of the studies. Numbers of exposed cases and controls are given.

| Author, year of publication, country, reference number                            | No. of cases | No. of controls | OR  | 95% CI  |
|---|--------------|-----------------|-----|---------|
| Inskip et al., 2001, USA [23]   | 40           | 358             | 0.8 | 0.5–1.4 |
| Lönn et al., 2004, Sweden [32] <sup>a</sup>                                       | 89           | 356             | 1.0 | 0.6–1.5 |
| Christensen et al., 2004, Denmark [33] <sup>a</sup>                               | 45           | 97              | 0.9 | 0.5–1.6 |
| Schoemaker et al., 2005, Denmark, Finland, Sweden, Norway, Scotland, England [34] | 360          | 1934            | 0.9 | 0.7–1.1 |
| Hardell et al., 2006, Sweden [11]   | 130          | 900             | 1.7 | 1.2–2.3 |
| Takebayashi et al., 2006, Japan [35]  | 51           | 192             | 0.7 | 0.4–1.2 |
| Klaeboe et al., 2007, Norway [31] <sup>a</sup>                                    | 22           | 227             | 0.5 | 0.2–1.0 |
| Schlehofer et al., 2007, Germany [36]   | 29           | 74              | 0.7 | 0.4–1.2 |
| Hours et al., 2007, France [30]   | 58           | 123             | 0.9 | 0.5–1.6 |
| Meta-analysis   | 668          | 3581            | 1.0 | 0.8–1.1 |

<sup>a</sup> Not included in meta-analysis because already part of pooled data in Schoemaker et al., 2005 [34].

Long-term exposure to RF fields from mobile phones and brain tumor risk is of importance to evaluate, not the least since the use of cellular phones is globally widespread with high prevalence among almost all age groups in the population. In the following we discuss mobile phone use and the association with brain tumors, but also other tumor types that have been studied. Recently, we published a detailed review of studies on brain tumors [14] followed by meta-analyses of published studies regarding glioma, acoustic neuroma and meningioma [15]. We have now recalculated these results with the addition of two new recently published articles from the Interphone study group [16,17]. Studies from individual countries were only included in the meta-analyses if they were not also included in the joint publications for several countries. For odds ratio (OR) and 95% confidence interval (CI) we used fixed effects model as in the recent publication by Kundi [18]. The analyses were done using Stata/SE 10 (Stata/SE 10 for Windows; StataCorp., College Station, TX).

One case-control study was excluded since no separate data were presented for glioma, acoustic neuroma or meningioma [19], and another since no overall data on acoustic neuroma were published, only for some time periods without results for  $\geq 10$  year latency period [20].

Due to several methodological limitations a Danish cohort study on "mobile phone subscribers" [21] is not possible to include in the meta-analysis, and the same methodological shortcomings prevail in the published updated cohort [22]. In the following only a short overview of the results for brain tumors is given, since we have discussed these issues in more detail elsewhere [14,15]. The other tumor types that have been studied are salivary gland tumors, non-Hodgkin lymphoma (NHL), testicular cancer, eye melanoma and facial nerve tumor.

## 2. Glioma

Glioma is a malignant type of brain tumor and comprises about 60% of all central nervous system tumors. The highly malignant glioblastoma multiforme, with poor survival, is included in this group.

Eleven case-control studies present results for glioma [12,17,23–31]. Of these eight [17,25–31] were part of the Interphone study and four of these [25–27,31] were included in a pooled-analysis with additional data for Finland [29]. The results are presented in Table 1. Overall no decreased

Table 4

Odds ratios (ORs) and 95% confidence intervals (CIs) from four case-control studies on acoustic neuroma including meta-analysis of the studies using  $\geq 10$  year latency period. Numbers of exposed cases and controls are given.

| Study  | Total                 |     |          | Ipsilateral           |     |         | Contralateral         |     |         |
|--|-----------------------|-----|----------|-----------------------|-----|---------|-----------------------|-----|---------|
|  | No. of cases/controls | OR  | 95% CI   | No. of cases/controls | OR  | 95% CI  | No. of cases/controls | OR  | 95% CI  |
| Lönn et al., 2004, Sweden, $\geq 10$ years [32] <sup>a</sup>                                       | 14/29                 | 1.8 | 0.8–4.3  | 12/15                 | 3.9 | 1.6–9.5 | 4/17                  | 0.8 | 0.2–2.9 |
| Christensen et al., 2004, Denmark, $\geq 10$ years [33] <sup>a</sup>                               | 2/15                  | 0.2 | 0.04–1.1 | –                     | –   | –       | –                     | –   | –       |
| Schoemaker et al., 2005, Denmark, Finland, Sweden, Norway, Scotland, England, $\geq 10$ years [34] | 47/212                | 1.0 | 0.7–1.5  | 31/124                | 1.3 | 0.8–2.0 | 20/105                | 1.0 | 0.6–1.7 |
| Hardell et al., 2006, Sweden, $>10$ years [11]   | 20/99                 | 2.9 | 1.6–5.5  | 10/28                 | 3.5 | 1.5–7.8 | 6/29                  | 2.4 | 0.9–6.3 |
| Meta-analysis  | 67/311                | 1.3 | 0.97–1.9 | 41/152                | 1.6 | 1.1–2.4 | 26/134                | 1.2 | 0.8–1.9 |

<sup>a</sup> Not included in meta-analysis because already part of pooled data in Schoemaker et al., 2005 [34].

or increased risk was found for glioma in the meta-analysis; OR = 1.0, 95% CI = 0.9–1.1.

Results for 10 year latency period are presented in Table 2. Six studies [12,25–29] gave such information and three [25–27] of these were also part of the publication by Lähkola et al. [29]. The meta-analysis yielded significantly increased risk for glioma with OR = 1.3, 95% CI = 1.1–1.6 increasing to OR = 1.9, 95% CI = 1.4–2.4 for ipsilateral exposure. The latter results were based on 118 exposed cases and 145 exposed controls. Regarding contralateral exposure to microwaves from mobile phones a lower risk was calculated, OR = 1.2, 95% CI = 0.9–1.7 ( $n$  = 93 cases, 150 controls). It should be noted that in the study by Takebayashi et al. [17] analyses of maximum microwave energy absorbed at the location of the tumor gave OR = 1.6, 95% CI = 0.6–4.2 related to the highest quartile of cumulative phone time weighted by maxSAR and OR = 5.8, 95% CI = 0.96–36 for subjects with cumulative maxSAR-hour of  $\geq 10$  W/kg-h.

### 3. Acoustic neuroma

These tumors are benign and do not undergo malignant transformation. They tend to be encapsulated and grow in relation to the auditory and vestibular portions of nerve VIII. They are slow growing tumors initially in the auditory canal, but gradually grow out into the cerebellopontine angle, where they come into contact with vital brain stem centers.

Nine case-control studies have been published [11,23,30–36], see Table 3. Seven [30–36] were part of the Interphone study and three [31–33] were included in the publication by Schoemaker et al. [34]. Analysis of the total material yielded OR = 1.0, 95% CI = 0.8–1.1 increasing to 1.3, 95% CI = 0.97–1.9 using 10 year latency period, Table 4. For ipsilateral exposure OR increased further to 1.6, 95% CI = 1.1–2.4, whereas contralateral exposure gave a non-significantly increased risk, OR = 1.2, 95% CI = 0.8–1.9.

### 4. Meningioma

Meningioma arises from the pia or arachnoid, which are the covering layers of the central nervous system. The majority are benign tumors that are encapsulated and well-demarcated from surrounding tissue.

Regarding meningioma results have been published from nine case-control studies, Table 5 [11,16,17,23,25,26,28,30,31]. Of these, seven [16,17,25,26,28,30,31] were part of the Interphone studies. The Lähkola et al. study [16] included three separately published Interphone studies [25,26,31]. The meta-analysis in Table 5 gave a significantly reduced OR = 0.9, 95% CI = 0.8–0.9. These results were mainly caused by the findings in the Interphone study [16] with the largest numbers of cases and controls yielding OR = 0.8, 95% CI = 0.7–0.9 in that study.

Using 10 year latency period OR was close to unity and somewhat increased for ipsilateral exposure, OR = 1.3, 95% CI = 0.9–1.8, Table 6. Regarding contralateral exposure OR was non-significantly decreased to 0.8, 95% CI = 0.5–1.3. The results for laterality were based on only two studies [11,16].

### 5. Brain tumor risk in different age groups

We grouped cases and controls according to age when they started to use a mobile or a cordless phone [11,12]. Consistently we found the highest risk for those with first use <20 years age. Thus, for malignant brain tumors OR = 2.7, 95% CI = 1.3–6.0 was calculated for mobile phones and OR = 2.1, 95% CI = 0.97–4.6 for cordless phones. The corresponding results for benign brain tumors were OR = 2.5, 95% CI = 1.1–5.9 and OR = 0.6, 95% CI = 0.2–1.9, respectively. Previously, we published results for diagnosis of brain tumor in different age groups [37] and found highest OR = 5.9, 95% CI = 0.6–55 for ipsilateral use of analogue phones in the youngest age group 20–29 years at the time of diagnosis. Using a >5 years latency period increased the risk further.

### 6. Brain tumor risk for use of mobile phone in urban and rural areas

There is a difference in output power of digital mobile phones between urban and rural areas. Adaptive power control (APC) regulates power depending on the quality of the transmission. In rural areas with on average longer distance to the base station the output power level is higher than in urban areas with dense population and shorter distance to the base stations. We studied the risk for brain tumors in urban versus rural living from the data in our study with cases diagnosed January 1, 1997 to June 30, 2000 [38]. Regarding digital phones OR = 1.4, 95% CI = 0.98–2.0 was obtained for living in rural areas increasing to OR = 3.2, 95% CI = 1.2–8.4 with >5 years latency period. The corresponding results for living in urban areas were OR = 0.9, 95% CI = 0.8–1.2 and OR = 0.9, 95% CI = 0.6–1.4, respectively.

### 7. Salivary gland tumors

The salivary glands, especially the parotid gland, are targets for near-field microwave exposure during calls with wireless phones. A Finnish study reported OR = 1.3, 95% CI = 0.4–4.7 for those who had ever had a mobile phone subscription [24].

Results from three case-control studies have been published, one from Sweden, one from the Nordic countries and one from Israel. During the same period as our studies on brain tumors we performed a study on salivary gland tumors [39]. Our study included the whole Swedish pop-

Table 5

Odds ratios (ORs) and 95% confidence intervals (CIs) from nine case–control studies on meningioma including meta-analysis of the studies. Numbers of exposed cases and controls are given.

| Author, year of publication, country, reference number           | No. of cases | No. of controls | OR  | 95% CI  |
|--|--------------|-----------------|-----|---------|
| Inskip et al., 2001 (USA) [23]                                   | 67           | 358             | 0.8 | 0.5–1.2 |
| Lönn et al., 2005 (Sweden) [25] <sup>a</sup>                     | 118          | 399             | 0.7 | 0.5–0.9 |
| Christensen et al., 2005 (Denmark) [26] <sup>a</sup>             | 67           | 133             | 0.8 | 0.5–1.3 |
| Schüz et al., 2006 (Germany) [28]                                | 104          | 234             | 0.8 | 0.6–1.1 |
| Hardell et al., 2006 (Sweden) [11]                               | 347          | 900             | 1.1 | 0.9–1.3 |
| Klaeboe et al., 2007 (Norway) [31] <sup>a</sup>                  | 96           | 227             | 0.8 | 0.5–1.1 |
| Hours et al., 2007 (France) [30]                                 | 71           | 80              | 0.7 | 0.4–1.3 |
| Lahkola et al., 2008 (Denmark, Norway, Finland, Sweden, UK) [16] | 573          | 1696            | 0.8 | 0.7–0.9 |
| Takebayashi et al., 2008, Japan [17]                             | 55           | 118             | 0.7 | 0.4–1.2 |
| Meta-analysis  | 1217         | 3386            | 0.9 | 0.8–0.9 |

<sup>a</sup> Not included in meta-analysis because already part of pooled data in Lahkola et al., 2008 [16].

ulation. Cases were recruited by using the regional cancer registries, and most had a malignant disease. They were diagnosed during 1994–2000, but with some variation for the different medical regions in Sweden. Population based controls were used as reference group. The questionnaire was answered by 267 (91%) of the cases and 750 (92%) of the controls. Of the cases 245 had a cancer diagnosis. Overall no association was found; analogue phones yielded OR=0.9, 95% CI=0.6–1.4, digital OR=1.0, 95% CI=0.7–1.5 and cordless phones OR=1.0, 95% CI=0.7–1.4. No effect of tumor induction period was found, although regarding >10 year latency period only 6 cases had used an analogue phone, OR=0.7, 95% CI=0.3–1.7, whereas no case had used a digital or cordless phone with that latency period. The results did not change significantly for ipsilateral or contralateral tumors.

The Nordic part of the Interphone case–control study of an association between use of mobile phones and parotid gland tumors was published in 2006 [40]. Detailed information about mobile phone use was obtained from 60 (85%) cases with malignant tumor, 112 (88%) with benign tumor and 681 (70%) controls. Regular mobile phone use gave OR=0.7, 95% CI=0.4–1.3 for malignant tumors and OR=0.9, 95% CI=0.5–1.5 for benign parotid gland tumors. For ipsilat-

eral mobile phone use a latency period of  $\geq 10$  year yielded OR 0.7, 95% CI=0.1–5.7 for malignant tumors ( $n=1$ ) and OR=2.6, 95% CI=0.9–7.9 for benign tumors ( $n=6$ ). Contralateral use was reported by one case with benign tumor and no case with malignant tumor in the same latency group.

As part of the Interphone study results on parotid gland tumor were reported from Israel [41]. It included 402 benign and 58 malignant incident cases, total 460 (87%) of 531 eligible for the time period 2001–2003. Population based matched controls were used, in total 1266 (66%) out of 1920 eligible subjects. Thirteen cases had a latency period of  $\geq 10$  year, which gave OR=0.9, 95% CI=0.4–1.8. No significantly increased risk was found for duration of use;  $\geq 10$  year yielded OR=1.0, 95% CI=0.5–2.1. However, for cumulative number of calls >5479 OR=1.6, 95% CI=1.1–2.2 was found for ipsilateral and both ears used equally, whereas contralateral use gave OR=0.8, 95% CI=0.5–1.2. Similarly, cumulative call time >266.3 h yielded OR=1.5, 95% CI=1.1–2.1; contralateral use gave OR=0.8, 95% CI=0.6–1.3.

In the meta-analysis using 10 year latency period no overall increased risk was found, OR=0.8, 95% CI=0.5–1.4, but for ipsilateral use it increased to OR=1.7, 95% CI=0.96–2.9, whereas contralateral use gave OR=0.4, 95% CI=0.2–1.2, Table 7.

Table 6

Odds ratios (ORs) and 95% confidence intervals (CIs) from five case–control studies on meningioma including meta-analysis of the studies using  $\geq 10$  year latency period. Numbers of exposed cases and controls are given.

| Study  | Total                 |     |          | Ipsilateral           |     |          | Contralateral         |     |          |
|--|-----------------------|-----|----------|-----------------------|-----|----------|-----------------------|-----|----------|
|  | No. of cases/controls | OR  | 95% CI   | No. of cases/controls | OR  | 95% CI   | No. of cases/controls | OR  | 95% CI   |
| Lönn et al., 2005, Sweden, $\geq 10$ years [25] <sup>a</sup>         | 12/36                 | 0.9 | 0.4–1.9  | 5/18                  | 1.3 | 0.5–3.9  | 3/23                  | 0.5 | 0.1–1.7  |
| Christensen et al., 2005, Denmark, $\geq 10$ years [26] <sup>a</sup> | 6/8                   | 1.0 | 0.3–3.2  | –                     | –   | –        | –                     | –   | –        |
| Schüz et al., 2006, Germany, $\geq 10$ years [28]                    | 5/9                   | 1.1 | 0.4–3.4  | –                     | –   | –        | –                     | –   | –        |
| Hardell et al., 2006, Sweden, >10 years [11]                         | 38/99                 | 1.5 | 0.98–2.4 | 15/28                 | 2.0 | 0.98–3.9 | 12/29                 | 1.6 | 0.7–3.3  |
| Lahkola et al., 2008 (Denmark, Norway, Finland, Sweden, UK) [16]     | 73/212                | 0.9 | 0.7–1.3  | 33/113                | 1.1 | 0.7–1.7  | 24/117                | 0.6 | 0.4–1.03 |
| Meta-analysis  | 116/320               | 1.1 | 0.8–1.4  | 48/141                | 1.3 | 0.9–1.8  | 36/146                | 0.8 | 0.5–1.3  |

<sup>a</sup> Not included in meta-analysis because already part of pooled data in Lahkola et al., 2008 [16].

Table 7

Odds ratios (ORs) and 95% confidence intervals (CIs) from three case-control studies on salivary gland tumors including meta-analysis of the studies using  $\geq 10$  year latency period.

| Study  | Total                 |     |         | Ipsilateral           |     |          | Contralateral         |                |                |
|--|-----------------------|-----|---------|-----------------------|-----|----------|-----------------------|----------------|----------------|
|  | No. of cases/controls | OR  | 95% CI  | No. of cases/controls | OR  | 95% CI   | No. of cases/controls | OR             | 95% CI         |
| Hardell et al., 2004, Sweden, $>10$ years [39]             | 6/35                  | 0.7 | 0.3–1.7 | 5/13                  | 1.5 | 0.5–4.2  | 1/15                  | 0.3            | 0.03–2.1       |
| Lönn et al., 2006, malignant, Sweden, $\geq 10$ years [40] | 2/36                  | 0.4 | 0.1–2.6 | 1/23                  | 0.7 | 0.1–5.7  | 0/19                  | – <sup>a</sup> | – <sup>a</sup> |
| Lönn et al., 2006, benign, Sweden, $\geq 10$ years [40]    | 7/15                  | 1.4 | 0.5–3.9 | 6/9                   | 2.6 | 0.9–7.9  | 1/9                   | 0.3            | 0.0–2.3        |
| Sadetzki et al., 2007, Israel, $\geq 10$ years [41]        | 13/26                 | 0.9 | 0.4–1.8 | 10/16                 | 1.6 | 0.7–3.7  | 3/10                  | 0.6            | 0.2–2.3        |
| Meta-analysis  | 28/112                | 0.8 | 0.5–1.4 | 22/61                 | 1.7 | 0.96–2.9 | 5/34                  | 0.4            | 0.2–1.2        |

<sup>a</sup> Not included in meta-analysis because OR could not be estimated.

## 8. Non-Hodgkin lymphoma

The incidence of NHL increased since the 1960s in Sweden as well as in many western countries with reliable cancer registries. This trend has levelled off since the 1990s, and decreasing exposure to environmental contaminants such as PCBs and dioxins, and also certain pesticides has been postulated to be one explanation [42,43]. As part of a large case-control study on NHL, mainly on exposure to pesticides [44], also questions on the use of wireless phones were included. The study covered the time period December 1, 1999 to April 30, 2002. The questionnaire was answered by 910 (91%) cases and 1016 (92% controls). The majority of the cases had B-cell NHL and we did not find any association with use of wireless phones [45]. Regarding T-cell NHL ( $n=53$ ) we observed somewhat increased risks; use of analogue phone gave OR = 1.5, 95% CI = 0.6–3.7, digital phone OR = 1.9, 95% CI = 0.8–4.8 and cordless phone OR = 2.5, 95% CI = 1.1–5.6. For certain subtypes of T-cell NHL, the cutaneous and leukemia types, the risks increased further for analogue phone to OR = 3.4, 95% CI = 0.8–15, digital phone to OR = 6.1, 95% CI = 1.3–30, and cordless phone to OR = 5.5, 95% CI = 1.3–24. These results were, however, based on low numbers.

A study from USA included 551 NHL cases and 462 frequency matched controls [46]. Among regular mobile phone users NHL risk was not significantly associated with minutes per week, duration, cumulative lifetime or years of first use. However, total time  $>8$  years gave OR = 1.6, 95% CI = 0.7–3.8. The risk increased with number of years, and was significant for the not specified group of NHL after  $\geq 6$  years use yielding OR = 3.2, 95% CI = 1.2–8.4.

## 9. Testicular cancer

An increasing incidence of testicular cancer has been noted in most western countries during the recent decades. It is the most common cancer type in young men and is

not regarded to be an occupational disease. Cryptorchidism is an established risk factors, but also perinatal exposure to persistent organic pollutants with hormone activity has been suggested to be another risk factor [47,48]. There has been concern in the population that use of mobile phones might be a risk factor for testicular dysfunction. We performed a case-control study mainly on the use of PVC plastics as risk factor for testicular cancer [49], and included in the questionnaire also questions on the use of wireless phones. The results were based on answers from 542 (92%) cases with seminoma, 346 (89%) with non-seminoma and 870 (89%) controls [50]. Overall no association was found [50]. Only 13 cases with seminoma had used an analogue phone  $>10$  years yielding OR = 2.1, 95% CI = 0.8–5.1 and one case with non-seminoma; OR = 0.3, 95% CI = 0.04–2.6. No case had used a digital or cordless phone with latency period  $>10$  years. OR did not increase with cumulative use in hours for the different phone types. Regarding use of mobile phone in the stand by mode border line significance was found for seminoma, OR = 1.3, 95% CI = 1.03–1.7, but not for non-seminoma; OR = 0.9, 95% CI = 0.7–1.3. For different localisations during stand by, highest risk was found for seminoma for keeping the phone in ipsilateral trousers pocket, OR = 1.8, 95% CI = 0.97–3.4 whereas contralateral pocket gave OR = 1.0, 95% CI = 0.5–2.0.

## 10. Malignant melanoma of the eye

Stang et al. [51] conducted a hospital- and population-based case-control study of uveal melanoma and occupational exposures to different sources of radiofrequency radiation. A total of 118 cases with uveal melanoma and 475 controls were included. Exposure to RF-transmitting devices was rated as (a) no RF exposure, (b) possible exposure to mobile phones, or (c) probable/certain exposure to mobile phones. An elevated risk for exposure to RF-transmitting devices was reported. Exposure to radio sets gave OR = 3.0, 95% CI = 1.4–6.3 and probable/certain exposure to mobile

phones OR = 4.2, 95% CI = 1.2–14.5. The authors concluded that several methodologic limitations prevented their results from providing clear evidence on the hypothesized association.

The study was commented among others Johansen et al. [52]. In their cohort of mobile phone subscribers in Denmark no support for an association between mobile phones and ocular melanoma was found. However, as discussed elsewhere [14,15,18,55], there are several methodological limitations in the Danish cohort [21,22] that hamper the interpretation of their findings.

The paper by Stang et al. [51] has also been commented by Inskip [53] in an editorial, the main point being that missing from the paper is any consideration of occupational or recreational exposure to UV radiation.

### 11. Intratemporal facial nerve tumor

So far only one investigation has studied the risk of intratemporal facial nerve (IFN) tumor and the use of mobile phone [54]. A case-control approach was used with 18 patients with IFN tumors matched with controls ( $n = 192$ ) treated for other diseases, 51 patients treated for acoustic neuroma, 72 treated for rhinosinusitis, and 69 for dysphonia and gastroesophageal reflux. Risk of facial nerve tumorigenesis was compared by extent of mobile phone use. The OR of developing an IFN tumor was 0.6, 95% CI = 0.2–1.9 with any handheld mobile phone use and OR = 0.4, 95% CI = 0.1–2.1 for regular mobile phone use. However, they concluded that the short duration of use precludes definite exclusion as a risk for IFN tumor development. Certainly the cases were too few for a sound epidemiological study and it was not correct to include patients with acoustic neuroma in the reference group.

### 12. Discussion

A review on use of mobile phones and the association with brain tumors included all case-control studies that we have identified in the peer-review literature. Most studies have published data with rather short latency period and limited information on long-term users.

No other studies than from the Hardell group has published comprehensive results for use of cordless phones (DECT) [2–15]. As we have discussed in our publications it is pertinent to include also such use in this type of studies. Cordless phones are an important source of exposure to microwaves and they are usually used for a longer time period on daily basis as compared to mobile phones. Thus, to exclude such use, as was done in e.g. the Interphone studies, could lead to an underestimation of the risk for brain tumors from use of wireless phones.

We have discussed shortcomings in the Interphone studies in detail elsewhere [55]. Regarding glioma the Swedish

Interphone study reported 23 ORs in Table 2 in that publication [25] and 22 of these were <1.0 and one OR = 1.0. For meningioma all 23 ORs were <1.0, six even significantly so. These results indicate a systematic bias in the study unless use of mobile phones prevents glioma and meningioma, which is biologically unlikely. It should be noted that several of the overall ORs also in other Interphone studies were <1.0, some even significantly so. As an example, in the Danish Interphone study on glioma [26] all 17 ORs for high-grade glioma were <1.0, four significantly decreased. Also other Interphone studies reported ORs significantly <1.0, that is a protective effect or rather systematic bias in the studies [16,29,31].

Use of cellular telephones was mostly assessed by personal interviews in the Interphone studies. It is not described how these personal interviews were organized, a tremendous task considering that vast parts of Sweden from north to south had to be covered. In the sparsely populated and extended area in northern Sweden personal interviews must have meant lots of long distance traveling and imposed additional stress on the interviewers. No information was given in the articles on how or if this methodological problem was solved, for example were controls only included from more densely populated areas.

The interviews in the Interphone study were extensive and computer aided. It is likely that such an interview creates a stressful situation for a patient with a recent brain tumor diagnosis and operation. These patients, especially under pressure with a newly diagnosed brain tumor and possible surgery, often have difficulties remembering past exposures and inevitably have problems with concentration and may have problems with other cognitive shortcomings. In the Danish part of the Interphone study it was concluded that the patients scored significantly lower than controls due to recalling words (aphasia), problems with writing and drawing due to paralysis [26]. According to our experience a better option would have been to start with a mailed questionnaire, that can be answered by the patient during a period of more well-being, if necessary this can be complemented by a telephone interview. After surgery it is easier to answer a questionnaire at home, also with the possibility to check phone bills to verify the use. This procedure has the additional advantage that it can be accomplished without disclosure during the data collection, whether a person is a case or a control. Certainly, knowing if it was a case or a control that was interviewed in the Interphone study may have introduced observational bias.

It has been argued that recall bias might be introduced in case-control studies on cancer patients, since the patients would be more prone to find a cause for their disease than the controls. However, the contrary is often the situation since patients do not want to blame themselves for their disease. In one article we presented data on the patients own assumptions of causes of their brain tumor [5]. Of 1429 cases only two expressed concern about mobile phones and no about cordless

phones. Interestingly, cases with a previous cancer diagnosis reported lower frequency for use of wireless phones than those with no previous cancer. No interviewer bias could be demonstrated when exposure data in the questionnaire were compared before and after phone interviews [5].

The diagnosis of tumor type as well as grading is based on histopathology. X-ray investigation or MR alone is insufficient. Of the 371 cases with glioma in the Swedish Interphone study [25] histopathology examination of the tumor was available for 328 (88%) cases, and for 225 (82%) of the meningioma cases. Thus, it is possible that cases without histology confirmation of the diagnosis may have had another type of brain tumor or even brain metastases. Such misclassifications inevitably bias the result towards unity. It is remarkable that 345 glioma cases were stratified according to grade I–IV, although histopathology was available only for 328 cases. In our studies on brain tumors we have histopathology verification of all of the diagnoses. Also, the total number of included cases [25] is not completely consistent with those reported to the Swedish Cancer Registry as we have discussed elsewhere [55]. The study included cases from neurosurgery, oncology and neurology clinics as well as regional cancer registries in the study areas.

Among the controls in the glioma and meningioma study 282 (29%) refused to participate [25]. Among some of these non-responders a short interview was made and only 34% reported regular use of a cellular telephone compared with 59% of the responders. If this discrepancy extends to the total group of non-responders the true percentage of mobile phone users in controls would be approximately 52%. Hence this figure would be lower than in glioma (58% exposed) and acoustic neuroma cases (60%). Only for meningioma with 43% exposed cases a lower percentage was reported, however, considering the sex ratio (women:men) for meningioma of about 2:1 a lower percentage of mobile phone users has to be expected due to the lower rate of users among women. It should be noted that a similar procedure in another Interphone study yielded similar results regarding mobile phone use among responders and non-responders [17].

It was discussed in a medical dissertation [56] that: 'Our Swedish study, that includes a large number of long-term mobile phone users, does not support the few previously reported positive findings, and does not indicate any risk increases neither for short-term or long-term exposures.' Considering the methodological shortcomings and that in contrast to the cited assertion of 'a large number of long-term users' the study subjects included only 25 glioma and 12 meningioma cases with long-term use, its conclusion seems to be going a long way beyond what can be scientifically defended.

It might be mentioned that this area of research seems to be controversial *per se* with unfounded statements [57], easily rebutted [58] and not supported by evolving scientific evidence [59]. Statements on no risk for brain tumors based on short-time use of mobile phones [60] might be considered in a larger context [61].

We included in our studies use of mobile or cordless phone 'any time' in the exposed group and made dose-response calculations based on number of hours of cumulative use. The unexposed group included also subjects with use of wireless phones with  $\leq 1$ -year latency period. On the contrary, mobile phone use in the Interphone studies was defined as 'regular use' on average once per week during at least 6 months, less than that was regarded as unexposed including also all use within  $<1$  year before diagnosis. This definition of 'regular use' seems to have been arbitrary chosen and might have created both observational and recall bias in the interpretation of such a definition.

Use of cordless phones was not assessed or not clearly presented in the Interphone studies, e.g. [25,28]. We found a consistent pattern of an association between cordless phones and glioma and acoustic neuroma [11,12]. It has been shown that the GSM phones have a median power in the same order of magnitude as cordless phones [62]. Moreover, cordless phones are usually used for longer calls than mobile phones [11,12]. Including subjects using cordless phones in the "unexposed" group in studies on this issue, as for example in the Interphone investigations, would thus underestimate the risk and bias OR against unity.

The case participation was good in our studies, 88% for cases with benign brain tumors, 90% for malignant brain tumor cases and 89% for the controls. On the contrary case participation varied from 37% to 93% and control participation from 42% to 75% in the Interphone studies. Obviously low participation rates for cases and controls might give selection bias and influence the results in the Interphone studies.

Methodological issues in the Interphone studies have been discussed elsewhere [14,15,18,55,63–65]. It was concluded that the actual use of mobile phones was underestimated in light users and overestimated in heavy users. Random recall bias could lead to large underestimation in the risk of brain tumors associated with mobile phone use. It was further suggested that selection bias in the Interphone study resulted in under selection of unexposed controls. Refusal to participate was related to less prevalent use of mobile phones, and this could result in a downward bias in estimates of the disease risk associated with mobile phone use. As discussed by Kundi [18] there was also interview lag time between cases and controls in the Interphone studies that might have been a source of bias due to the fast increase of mobile phone use during the study period. This could have resulted in underestimation of risk.

For salivary gland tumors the results were based on three case-control studies. In the 10 year latency period the meta-analysis gave an almost significantly increased risk for ipsilateral use of mobile phones, and a non-significantly decreased risk for contralateral use. These results were based on few cases. Regarding NHL and testicular cancer some subgroup analysis yielded increased risks, but these results were based on low numbers. Use of mobile phone increased the risk significantly for melanoma of the eye. The study on intratemporal facial nerve tumors is not informative since

it was based on few cases and included acoustic neuroma patients in the control group. It is concluded that all studies were hampered by low numbers of long-term users and need to be replicated for firm evidence of an association between use of mobile phones and these tumor types.

In summary our review yielded a consistent pattern of an increased risk for acoustic neuroma and glioma after >10 years mobile phone latency. Our studies showed also an association with use of cordless phones, an issue that has not been studied at all in most investigations or only rudimentary in two studies. We conclude that current standard for exposure to microwaves during mobile phone use is not safe for long-term exposure and needs to be revised.

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# Mobile phone base stations—Effects on wellbeing and health

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## Abstract

Studying effects of mobile phone base station signals on health have been discouraged by authoritative bodies like WHO International EMF Project and COST 281. WHO recommended studies around base stations in 2003 but again stated in 2006 that studies on cancer in relation to base station exposure are of low priority. As a result only few investigations of effects of base station exposure on health and wellbeing exist. Cross-sectional investigations of subjective health as a function of distance or measured field strength, despite differences in methods and robustness of study design, found indications for an effect of exposure that is likely independent of concerns and attributions. Experimental studies applying short-term exposure to base station signals gave various results, but there is weak evidence that UMTS and to a lesser degree GSM signals reduce wellbeing in persons that report to be sensitive to such exposures. Two ecological studies of cancer in the vicinity of base stations report both a strong increase of incidence within a radius of 350 and 400 m respectively. Due to the limitations inherent in this design no firm conclusions can be drawn, but the results underline the urgent need for a comprehensive investigation of this issue. Animal and in vitro studies are inconclusive to date. An increased incidence of DMBA induced mammary tumors in rats at a SAR of 1.4 W/kg in one experiment could not be replicated in a second trial. Indications of oxidative stress after low-level in vivo exposure of rats could not be supported by in vitro studies of human fibroblasts and glioblastoma cells.

From available evidence it is impossible to delineate a threshold below which no effect occurs, however, given the fact that studies reporting low exposure were invariably negative it is suggested that power densities around 0.5–1 mW/m<sup>2</sup> must be exceeded in order to observe an effect. The meager data base must be extended in the coming years. The difficulties of investigating long-term effects of base station exposure have been exaggerated, considering that base station and handset exposure have almost nothing in common both needs to be studied independently. It cannot be accepted that studying base stations is postponed until there is firm evidence for mobile phones.

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## 1. Introduction

Modern mobile telecommunication is based on a cellular system. Each cell is covered by a base station that keeps track of the mobile phones within its range, connects them to the telephone network and handles carry-over to the next base station if a customer is leaving the coverage area. Early mobile telecommunication systems had very large cells with tens of kilometers radius and were predominantly located along highways due to offering service mainly for car-phones. With the introduction of digital mobile phone systems cell sizes got much smaller and base stations were erected in densely

populated areas. The limited power of mobile phones made it necessary to reduce the distance to the customers. The cell size depends on (1) the radiation distance of the mobile phone; (2) the average number of connected calls; (3) the topographic characteristics of the covered area and the surrounding buildings, vegetation and other shielding objects; and (4) the type of antenna used. There are essentially three types of cells presently making up mobile telecommunication networks: (1) macro-cells in areas of average to low number of calls; (2) micro-cells in densely populated areas and areas with high telecommunication traffic density; (3) pico-cells within buildings, garages, etc. The types of antennas used, although hundreds of different models are operated, can be subdivided into: omni-directional antennas that radiate in all horizontal directions with the same power; sector antennas

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that radiate the main beam in one sector only but have varying aperture (usually 120° or 90°). These antennas can be mounted on masts (that sometimes are in the shape of trees for protection of landscape or are otherwise hidden), on the top of buildings, on pylons, and micro- and pico-cell antennas on various other places (walls of houses, shops, indoors, etc.). The width of the beam in vertical direction is typically 6°, but due to the presence of side lobes the actual pattern is more complicated.

Digital base stations of the second generation (GSM, TDMA) and third generation (UMTS, CDMA) have typically a nominal power for each channel of 10–20 W, micro- and pico-cells up to about 4 and 2 W, respectively. Due to the antenna gain the EIRP in the direction of the main beam is much greater (by a factor of  $10^{g/10}$ , where  $g$  is the antenna gain in dB, typically between 40 and 60). Most base stations of the second generation operate with two channels, one broadcast control channel (BCCH, channel used for transmitting information about the network, the location area code, frequencies of neighboring cells, etc.) and one traffic channel (TCH, channel used for transmission of calls), for third generation systems, due to code division multiplexing, control information needed for the maintenance of the system is at present transmitted together with the actual information (calls, pictures, etc.) within one broad-band channel. GSM systems operate the BCCH with all time slots occupied and therefore at maximal power, whereas TCH has as many time slots active as necessary to operate all active transmission not covered by the BCCH. Field strength at ground level depends on the characteristics of the antenna. Because the main beam reaches ground level typically in 50–200 m distance, in case of free sight to the antenna, maximum field strength is reached at that distance. However, due to the side lobes ups and downs of field strength occur as one approach the base station. In areas where objects are shadowing the beams, patterns are still more complex because of diffraction and reflection and multi-path propagation with constructive as well as destructive interference.

Free field propagation from the antenna along the main beam follows the law:  $P(x) = \text{EIRP}/(4\pi \cdot x^2)$ , with  $P(x)$  the power flux density in  $x$  meters distance and EIRP the equivalent isotropic radiated power of the antenna. Significant deviations from this expectation occur due to the side lobes, presence of interfering objects, differences in vertical beam width, and variations in the number of active transmissions. For these reasons distance to the antenna is a poor proxy for exposure level.

Since the early 1990s tens of thousands of base stations have been erected in countries where digital networks were introduced. While older systems with their low number of base stations have hardly received public attention, the vast increase in base stations has led to public concerns all over the world. Anecdotal reports about various effects on wellbeing and health have led also to an increased awareness of physicians [1,2] and increased research efforts have been demanded [3]. Despite these professional and public con-

cerns, the WHO International EMF Project has discouraged research into effects of base stations, because it deemed research into effects of mobile phones of higher priority. This position was changed in 2003 when the new research agenda recommended studies around base stations. In 2006 it was again stated that research into potential health effects of base station is of low priority [4].

Due to these circumstances only very few investigations of effects of base stations on wellbeing and health exist. In addition some experimental studies have been conducted, most of which address the problem of short-term effects on complaints and performance.

The following review summarizes available evidence and critically assesses the investigations as to their ability to support or dismiss a potential effect of microwave exposure from base stations on wellbeing and health.

## 2. Epidemiological investigations

### 2.1. Wellbeing and performance

Santini et al. [5,6] report results of a survey in France to which 530 individuals (270 men and 260 women) responded. Study subjects were enrolled through information given by press, radio, and website, about the existence of a study on people living near mobile phone base stations. Frequency for each of 18 symptoms was assessed on a 4 level scale (never, sometimes, often, and very often). Participants estimated distance to the base station using the following categories: <10 m, 10–50 m, 50–100 m, 100–200 m, 200–300 m, >300 m. For comparison of prevalence of symptoms >300 m served as reference category. For all symptoms a higher frequency of the categories 'often' or 'very often' was found at closer (self-reported) distance to the base station. Fatigue, headaches, and sleeping problems showed highest relative increase. Due to a less than optimal statistical analysis comparing each distance category separately with the reference category the overall response pattern can only be assessed qualitatively. Fig. 1 shows relative prevalence averaged over all symptoms as a function of self-reported distance to the antenna. Interestingly the function is not monotonous but shows, after an initial drop, an increase at a distance of 50–100 m. Because of the fact that in many cases this is the distance at which the main beam reaches ground level this may indicate a relationship to actual exposure levels.

This study was a first attempt to investigate a potential relationship between exposure to base station signals and health and has, therefore, several shortcomings: (1) participants selected themselves into the study group by responding to public announcements; (2) distance was self-reported and no attempt was made to validate these reports (a German cross-sectional study in over 30,000 households revealed that more than 40% did not know they were living in the vicinity of a base station [7]); (3) no assessment of subjects' concerns about the base station; and (4) no measurement or calcula-

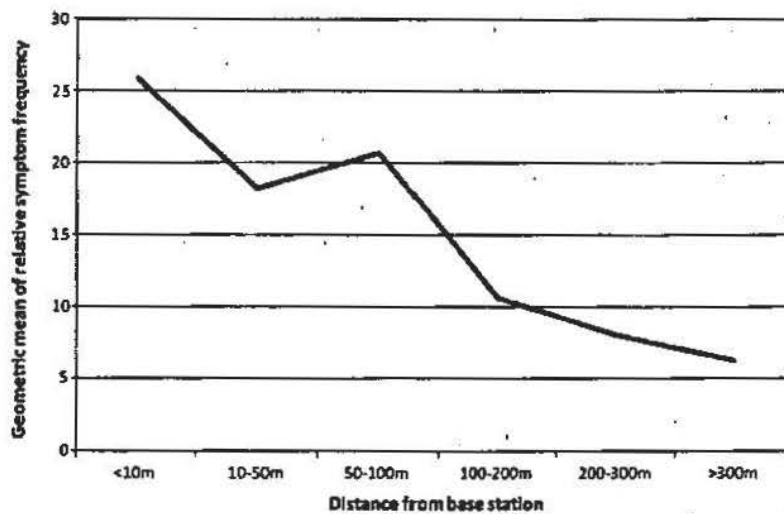


Fig. 1. Relative symptom frequency averaged over all 33 reported symptoms from Santini et al. [5] as a function of distance from base station.

tion of actual exposure. Although selection bias and wrong estimation of distance to the base station could have led to a spuriously increased prevalence of symptoms, the pattern of symptom frequency as a function of distance is intriguing and suggests that part of the increased symptom prevalence could be due to exposure because people do not know the typical pattern of field strengths found in the vicinity of base stations.

A Spanish version of the questionnaire as applied in the French study was distributed in La Nora, a small town in Murcia, Spain, to about 145 inhabitants [8]. Overall 101 questionnaires (from 47 men and 54 women) were included in the analyses. Electric field strength in the frequency range 1 MHz to 3 GHz was measured in the bedrooms of the participants. Data were analyzed in two different ways: first subjects were subdivided into those living less than 150 m from the base station and a second group living more than 250 m away (according to self-reports); the average exposure level of the first group was 1.1 mW/m<sup>2</sup>, and of the second group 0.1 mW/m<sup>2</sup>; self-reported symptom severity was compared across these groups. The second method correlated log transformed field strengths with symptom scores. The majority of symptoms showed a relationship both by comparison of the contrast groups according to distance from the base station as well as when correlated to measured field strength. Strongest effects were observed for headaches, sleep disturbances, concentration difficulties, and discomfort.

In contrast to the French investigation the study has assessed actual exposure by short-term measurements in the bedrooms of participants. The fact that both, reported distance as well as measured field strength, correlated with symptom severity supports the hypothesis of an association between microwaves from the base station and wellbeing. However, because subjects knew that the intention of the study was to assess the impact of the base station there is a potential for bias. Also concerns of the participants about effects of the base station on health were not assessed. Furthermore, method of selection of participants was not reported.

In a cross-sectional study in the vicinity of 10 GSM base stations in rural and urban areas of Austria, Hutter et al. [9] selected 36 households randomly at each location based on the characteristics of the antennas. Selection was done in such a way as to guarantee a high exposure gradient. Base stations were selected out of more than 20 locations based on the following criteria: (1) at least 2 years operation of the antenna; (2) no protest against it before or after erection; (3) no nearby other base station; (4) transmission only in the 900 MHz frequency band. (The last two criteria were not fully met in the urban area.) In order to minimize intervention of interviewers all tests and questionnaires were presented on a laptop computer and subjects fulfilled all tasks on their own. Wellbeing was assessed by a symptoms list (v. Zerssen scale), sleeping problems by the Pittsburgh sleeping scale. In addition several tests of cognitive performance were applied. Concerns about environmental factors were inquired and sources of EMF exposure in the household were assessed as well. It was not disclosed to the subjects that the study was about the base station, but about environmental factors in general. Among other measurements high-frequency fields were assessed in the bedrooms. From the measured field strength of the BCCH maximum and minimum exposure to the base station signals were computed. In addition overall power density of all high-frequency fields was measured. Results of measurements from 336 households were available for analysis. Exposure from the base station was categorized into three ranges: below 0.1 mW/m<sup>2</sup>, between 0.1 and 0.5 mW/m<sup>2</sup>, and above 0.5 mW/m<sup>2</sup>. Cognitive performance tended to be better at higher exposure levels and was statistically significant for perceptual speed after correction for confounders (age, gender, mobile phone use, and concerns about the base station). Subjective symptoms were generally more frequent at higher exposure levels and statistically increased prevalence was found for headaches, cold hands or feet, and concentration difficulties. Although participants reported more sleeping problems at higher exposure

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levels, this effect was removed after controlling for concerns about the base station.

Despite limitations inherent in the cross-sectional study design the methodological problems mentioned in the French and Spanish investigations were avoided. Authors conclude: "The results of this study indicate that effects of very low but long lasting exposures to emissions from mobile telephone base stations on wellbeing and health cannot be ruled out. Whether the observed association with subjective symptoms after prolonged exposure leads to manifest illness remains to be studied."

A study in employees working within or opposite a building with GSM base station antennas on the roof was reported by Abdel-Rassoul et al. [10]. The investigation took place in Shebin El-Kom City, Menoufiya Governorate, Egypt, where the first mobile phone base station was erected in 1998 on a building for agricultural professions. Overall 37 subjects working within this building and 48 subjects working in the agricultural directorate about 10 m opposite the building were considered exposed. A control group, working in another building of the agricultural administration located approximately 2 km away, consisted of 80 persons. Participants completed a structured questionnaire assessing educational and medical history. A neurological examination was performed and a neurobehavioral test battery (tests for visuomotor speed, problem solving, attention and memory) was presented. The combined exposed groups were compared to the control group that was matched by sex, age and other possible confounders. Statistical analysis accounted for these variables. Further comparisons were performed between subjects working in the building with the base station on the roof and those opposite. Exposed subjects performed significantly better in two tests of visuomotor speed and one test of attention, in two other tests the opposite was the case. The prevalence of headaches, memory problems, dizziness, tremors, depressive symptoms, and sleep disturbances was significantly higher among exposed inhabitants than controls. Measurements conducted 3 years before the investigation revealed compliance with the Egyptian standard (80 mW/m<sup>2</sup>) with values between 27 and 67 mW/m<sup>2</sup>, but locations of the measurements were not specified.

Like in the study of Hutter et al. [9] it was not disclosed to the participants that the study was about the base station. An important aspect is studying employees that occupy the area of exposure for 8–16 h a day. Several possible confounders (age, sex, education, smoking, and mobile phone use) were considered and did not change the reported results. Other factors like stressful working conditions, indoor pollutants and other attributes of the work place were not assessed and might have had an effect on the reported symptoms. Although no recent measurements were available it can be assumed that both, subjects working within the building as well as those opposite the building with the base station are exposed at comparatively high levels. The picture of one antenna shown in the article indicates that the panel is slightly uptilted. It can be assumed that the sidelobes of the antenna are directed

downwards into the building below the base station as well as into the opposite building. Measurements in Germany revealed that, in contrast to a general belief that there is no significant exposure in buildings below a base station antenna, the field strength in buildings below an antenna is almost equal to field strength in opposite buildings.

An experimental field trial was conducted in Bavaria [11] during three months before an UMTS antenna on a governmental building started operation. Based on a random sequence the antenna was turned on or off one, two, or three days in a row during 70 working days in winter 2003. Conditions were double-blind since neither the experimenters nor the participants knew whether the antenna was on or off. This was guaranteed by software manipulation of the antenna output that prohibited UMTS mobile phones from contacting the base station and by locating the computer controlling the antenna in a sealed room. The UMTS antenna operated at a mean frequency of 2167.1 MHz. The protocol has not been specified, but considering that no real transmission occurred it is assumed that only the service channel was used. The antenna had a down-tilt of 8° expected to result in rather high exposure within the building. Measured electric field strength in the rooms of the participants varied between the detection limit of the field probe (0.05 V/m) and 0.53 V/m (corresponding to 0.75 mW/m<sup>2</sup>) with an average of  $0.10 \pm 0.09$  V/m (corresponding to 0.03 mW/m<sup>2</sup>). Participants should answer an online questionnaire on each working day they were in the office in the morning when they arrived and in the evening shortly before leaving. The questionnaire consisted of a symptom list with 21 items, and in the evening participants should state whether or not they considered the antenna has been on during this day and whether they considered, if they experienced any adverse effects, these effects due to the base station. From approximately 300 employees working in the building 95 (28 females, 67 males) that answered the questionnaire on at least 25% of the working days were included in the analysis.

None of the 21 symptoms showed a statistically significant difference between days on and days off. A more comprehensive analysis of the overall score across all 21 items applying a mixed model with subjects as random factor and autoregressive residuals revealed a tendency ( $p = 0.08$ ) for an effect of actual exposure on the difference between morning and evening values. Self-rated electrosensitivity had a significant effect on evening scores but did not affect difference scores. As expected, subjective rating of exposure had a significant influence both, on evening scores and score difference. Correct detection rate of base station transmission mode was 50% and thus equal to chance. No person was able to detect operation mode correctly on significantly more days than expected.

The study design was a great strength of this pilot investigation. It combined the advantages of a field trial with the rigorous control of exposure conditions in an experiment. However, there are a number of severe shortcomings too: first, no correction for actual exposure has been applied. As

stated above, exposure varied considerably within the building and some participants were not exposed at detectable levels at all. The resulting exposure misclassification leads to a bias towards the null hypothesis. Furthermore, it was not specified which UMTS protocol was actually transmitted. Another important limitation is the quite low exposure even in the offices with the highest levels. Problems with the statistical evaluation are indicated by a highly significant time factor suggesting insufficient removal of autocorrelation. Finally, the symptom list contains several items that were not implicated previously as related to exposure from base stations (e.g. back pain). Such items reduce the overall power to detect an effect of base station exposure.

A cross-sectional study based on personal dosimetry was conducted in Bavaria [12]. In a sample of 329 adults (173 females, 155 males, and 1 unknown) chronic and acute symptoms were assessed. Chronic symptoms were taken from the Freiburger Beschwerdeliste and acute symptoms from the v. Zerssen list. Symptoms assessed were headache, neurological symptoms, cardiovascular symptoms, concentration problems, sleeping disorders and fatigue. Participants wore a dosimeter (Maschek ESM 140) for 24 h on the upper arm on the side used for holding a phone (during the night the dosimeter was placed next to the bed). The dosimeter measured exposure in frequency bands including GSM 900 up- and down-link, GSM 1800 up- and down-link, UMTS, DECT and WLAN (2.45 GHz).

Acute symptoms at noon and in the evening were dichotomized and related to exposure during the previous 6 h (night time measurements were considered biased and not analyzed). Exposure was expressed in percent of the ICNIRP reference levels. Odds ratios for the different symptom groups were computed in relation to exposure subdivided into quartiles with the first quartile as reference. Similarly, dichotomized chronic symptoms were related to average day time exposure levels. None of the symptom groups was significantly related to exposure. Odds ratios for headaches and cardiovascular symptoms during the last 6 months were increased for all three tested exposure quartiles (for headaches odds ratios were: 1.7, 2.7, and 1.2 for 2nd to 4th quartile; for cardiovascular symptoms these figures were 1.4, 3.3, and 2.4). But none of these odds ratios was statistically significant. Acute symptoms at noon and in the evening showed a tendency for lower prevalence of fatigue at higher exposure levels. Odds ratios for headaches and concentration problems in the evening were increased at higher exposure levels in the afternoon but also these results were statistically not significant (odds ratios for headaches were 1.7, 1.6, 3.1 and for concentration problems 1.4, 2.0, 1.4 for 2nd to 4th quartile of afternoon exposure levels).

Exposure was low and ranged from a daytime average of 0.05 V/m (at or below the limit of determination) to 0.3 V/m (corresponding to 0.24 mW/m<sup>2</sup> power density). (In order to make results comparable to other investigations figures expressed in percent of ICNIRP reference levels were recalculated to field strengths and power densities). Quartiles for

daytime exposure were: up to 0.075 V/m, 0.075 to 0.087 V/m, 0.087 to 0.110 V/m, and 0.110 to 0.3 V/m. It can be seen that the first three quartiles are almost indiscernible with a ratio of the upper limit of the third and first quartiles of only 1.5.

Although the study of Thomas et al. [12] was the first one using personal dosimetry in the context of investigating effects of exposure to mobile phone base station signals on wellbeing it has not explored the potential of an almost continuous exposure measurement. Only average exposure was computed and the probably most important nighttime values were left out. A number of different exposure metrics should have been assessed, like duration of exposure above a certain limit, maximum exposure level, longest period below limit of determination, and variability of exposure levels to name but a few. Furthermore, prevalence of symptoms was so low that the power of the investigation to detect even substantially increased risks was inferior (less than 25%). Despite these shortcomings the study has its merits as a first step in using personal dosimetry. An earlier report of the group [13] with a comparison between two personal dosimeters (Maschek and Antennessa) demonstrated that improvements are necessary before personal dosimetry can be successfully used in epidemiological studies.

A large population-based cross-sectional study was conducted in the context of the German 'Mobile Phone Research Program' in two phases [7]. In the initial phase 30,047 persons from a total of 51,444 (58% response rate) who took part in a nationwide survey also answered questions about mobile phone base stations. Additionally a list of 38 health complaints (Frick's list) was answered. Distance to the nearest base station was calculated based on geo-coded data of residences and base stations. In the second phase, all respondents (4150 persons) residing in eight preselected urban areas were contacted. In total, 3526 persons responded to a postal questionnaire (85% response rate) including questions about health concerns and attribution of symptoms to exposures from the base station as well as a number of standardized questionnaires: the Pittsburgh Sleep Quality Index, the Headache Impact Test, the v. Zerssen list of subjective symptoms, the profile of mental and physical health (SF 36), and a short version of the Trier Inventory of Chronic Stress. Overall 1808 (51%) of those that responded to the questionnaire agreed to have EMF measurement taken in their homes. Results of the large survey from the first phase of the study revealed a fraction of 10% of the population who attributed adverse health effects to the base station. An additional 19% were generally concerned about adverse effects of mobile phone base stations. Regression analysis of the symptoms summary score on distance to the base station (less or more than 500 m) and attribution/concerns about adverse effects adjusted for possible confounders (age, gender, SES, region and size of community) revealed a small but significant increase of the symptom score at closer distance to the base station. Higher effects, however, were obtained for concerns about adverse effects of the base station (with higher scores for those concerned) and still higher effects for

those that attributed their health problems to exposures from mobile phone base stations. The latter result is only to be expected because attribution presupposes existence of symptoms and hence those with attribution must have higher scores than those without. Because effects of concerns/attribution were accounted for in the multivariate model, effect of distance to the base station is independent of these concerns or attributions. In the second phase measurements in the bedrooms revealed an overall quite low exposure to EMFs from the base station. Only in 34% of the households was the exposure above the sensitivity limit of the dosimeters of  $0.05 \text{ V/m}$  ( $\sim 7 \mu\text{W/m}^2$ ). On average power density was  $31 \mu\text{W/m}^2$  and the 99th percentile amounted to  $307 \mu\text{W/m}^2$ . A dichotomization at the 90th percentile (exposure above  $0.1 \text{ V/m}$ , corresponding to  $26.5 \mu\text{W/m}^2$ ) did not indicate any effect of exposure on the different outcome variables but effects of attribution on sleep quality and overall symptom score (v. Zerssen list).

This large study has a number of important advantages: it started from a representative sample of the German population with over 30,000 participants and the second phase with a regional subsample had a participation rate of 85%. Furthermore, several well-selected standardized tests were used in the second phase. Results of the first phase are essentially in line with the Austrian study of Hutter et al. [9]. Not only the fraction with attribution of health complaints to exposure from the base station (10%) is identical, but also the higher symptom score in proximity to the base station independent of concerns/attributions found in the previous study has been replicated. However, the study has also severe shortcomings, most notably: the failure to include a sufficient number of participants that can be considered as exposed to microwaves from the base station. Note that Hutter et al. [9] selected households based on the characteristics of the antennas in such a way as to guarantee a large exposure gradient. In the randomly selected households of the study by Blettner et al. [7] the 90th percentile used as cutoff was well below the median ( $\sim 100 \mu\text{W/m}^2$ ) of the earlier investigation and the 99th percentile was still below the level ( $500 \mu\text{W/m}^2$ ) that was found to increase the prevalence of several symptoms. Therefore it is unlikely that the investigation of the second phase could detect an effect if it occurs at levels consistent with those reported by Hutter et al. [9].

## 2.2. Cancer

Despite considerable public concerns that exposure to microwaves from mobile phone base stations could be detrimental to health and may, in particular, cause cancer, up to now only two studies of cancer in the vicinity of base stations applying basically an ecological design have been published.

In a Bavarian town, Neila, the physicians of the town conducted an epidemiological investigation [14] to assess a possible association between exposure to base station radiation and cancer incidence. The design used was an improved ecological one. Two study areas were defined: one within

a circle of 400 m radius around the only base stations (two that were located in close proximity to each other) of the town, and one area further than 400 m from the base stations. Within these defined areas streets were randomly selected (after exclusion of a street where a home for retired people was situated) and all general practitioners of the town that were active during the whole period of operation of the base stations (one base station started operation September 1993 the other December 1997) scanned their files for patients living in the selected streets. Overall 967 individuals were found, constituting approximately 90% of the reference population. The study period 1/1994 to 3/2004 was subdivided into two segments: The first 5 years of operation of the base station (1994 through 1998) and the period from the sixth year, 1999, until 3/2004. Among the identified individuals 34 incident cases of cancer (excluding non-melanoma skin cancer) were found. Assessment of cancer cases was assumed to be complete and all cases were verified histologically and by hospital discharge letters (note that there is no cancer registry in Bavaria). Age distribution was similar in the two areas with a mean age of 40.2 years in both, the area within 400 m of the base station and the area further apart. Crude annual cancer incidence in the first 5 years after start of operation of the base station was  $31.3 \times 10^{-4}$  and  $24.7 \times 10^{-4}$  in the closer and farther area, respectively. In the second period these figures were  $76.7 \times 10^{-4}$  and  $24.7 \times 10^{-4}$ . The age and gender adjusted expected value of incident cancer cases in the study population based on data from Saarland, a German county with a cancer registry, is  $49 \times 10^{-4}$ . In the second period cancer incidence in the area within 400 m of the base station was significantly elevated, both, compared to the area further away as well as compared to the expected background incidence. The incidence in the region further apart was reduced but not significantly when compared to the expected value.

Although this so-called Neila-study applied an improved ecological design with a random selection of streets and inclusion of some information from selected individuals, it is still subject to potential bias because relevant individual risk factors could not be included in the analyses.

A similar though less rigorous study has been performed in Netanya, Israel. Wolf and Wolf [15] selected an area 350 m around a base station that came into operation 7/1996. The population within this area belongs to the outpatient clinic of one of the authors. The cohort within this area consisted of 622 people living in this area for at least 3 years at study onset, which was one year after start of operation of the base station and lasted for 1 year. Overall cancer incidence within the study area was compared to a nearby region, to the whole city of Netanya, and to national rates. In the second year after onset of operation 8 cancer cases were diagnosed in the study area. In the nearby area with a cohort size of 1222 individuals, 2 cases were observed. Comparison to the total population with an expected incidence of  $31 \times 10^{-4}$  indicates a pronounced increase in the study area with an incidence of  $129 \times 10^{-4}$ . Also against the whole town of Netanya an increased incidence was noted especially in women. In an

addendum authors noted that also in the subsequent year 8 new cases were detected in the study area while in the period 5 years before the erection of the base station 2 cases occurred annually. Spot measurements of high frequency fields were conducted in the homes of cancer cases and values between 3 and 5 mW/m<sup>2</sup> were obtained. Although these values are well below guideline levels, they are quite high compared to typical values measured in randomly selected homes [7].

Also in the case of the Netanya study lack of information on individual risk factors makes interpretation difficult. Furthermore, migration bias has not been assessed although only subjects were included that occupied the area for at least 3 years. The short latency after start of operation of the base station rules out an influence of exposure on induction period of the diseases. The substantial increase of incidence is also hardly explainable by a promotional effect.

### 3. Experimental studies

#### 3.1. Experiments in human sensitive and non-sensitive individuals

There are persons who claim to suffer from immediate acute as well as chronic effects on exposure to EMF and in particular to those from mobile phones or their base stations. Often these persons are called EMF hypersensitive (EHS). The preferred term agreed upon at a WHO workshop [16] was Idiopathic Environmental Intolerance with attribution to EMF (IEI-EMF). Indeed, it would be a misunderstanding to confuse EHS with allergic reactions; rather these persons react with different unspecific symptoms such as headaches, dizziness, loss of energy, etc. Whether these persons have actually the ability to tell the difference between situations with and without exposure to EMFs is an open question. In a recent review R  sli [17] concluded that "...the large majority of individuals who claim to be able to detect low level RF-EMF are not able to do so under double-blind conditions. If such individuals exist, they represent a small minority and have not been identified yet." However, it is important to differentiate between EMF sensitivity and sensibility [18]. Independent of the question whether or not there are individuals that sense the presence of low levels of EMFs such as those measured in homes near mobile phone base stations, there could well be an effect of such exposures on wellbeing and performance even under short-term exposure conditions. In several experimental investigations this question has been addressed by exposure of persons with self-reported symptoms and also in persons without known adverse reaction to an assumed exposure.

\* The first of these investigations was carried out by the Netherlands Organization for Applied Scientific Research (TNO) and published as a research report [19]. Two groups of persons were included in the experiment. One group consisted of individuals (25 females, 11 males) who have previously reported complaints and attributed them to GSM

exposure. The other group consisted of subjects without such complaints (14 females, 22 males). Four experimental conditions were applied in a double-blind fashion: Sham exposure, exposure to 945 MHz GSM, 1840 MHz GSM, and 2140 MHz UMTS. Each participant underwent sham exposure and two of the active exposure conditions. Sequence of exposure was balanced such that each active exposure condition was tested equally often at each of three experimental sessions. Each experimental session and a training session lasted for 45 min. All three experimental sessions and the training session were completed on one day for each participant. Both, for GSM and UMTS exposure, a base station antenna was used and a simulated base station signal was transmitted during sessions. For the GSM conditions a 50% duty cycle (4 slots occupied) was applied with pulses of peak amplitudes of 1 V/m (0.71 V/m effective field strength; corresponding to 1.3 mW/m<sup>2</sup>). For UMTS exposure a protocol was used with different low frequency components and an effective field strength of 1 V/m (corresponding to 2.7 mW/m<sup>2</sup>). During each session several performance tests were conducted and immediately after each session a wellbeing questionnaire was administered (an adapted version of the Quality-of-Life Questionnaire of Bulpitt and Fletcher [20] with 23 items).

Overall score of wellbeing was significantly reduced in both groups after the UMTS condition compared to sham exposure. Considering subscores anxiety symptoms, somatic symptoms, inadequacy symptoms, and hostility symptoms were increased in the groups of sensitive individuals whereas in the control group only inadequacy symptoms were increased after UMTS exposure compared to sham. No effects were found in the two GSM exposure conditions. Concerning cognitive performance both groups revealed significant exposure effects in almost all tests in different exposure conditions. In most of these tests reaction time was reduced except for one simple reaction time task.

This study had an enormous echo both in the media as well as in the scientific community because it was the first experimental investigation with very low exposure to base station like signals and in particular to UMTS signals, and because it was conducted by a highly respected research institution reporting systematic effects of exposure that seemed to support citizens initiatives claiming that base stations have adverse effects on wellbeing and health. Immediately doubts were expressed that results could be biased due to a faulty methodology. In fact, study design can be improved. First of all testing all exposure conditions on the same day has the advantage to reduce variance from between day differences but could cause transfer effects if biological reactions do not immediately terminate after end of exposure and start of the next condition. Also time-of-day effect from chronobiological variations could be superimposing the reactions from exposure. Such effects are sometimes not removed by balancing exposure conditions. Second, not all subjects were tested under all exposure conditions. The decision to reduce total experimental duration by presenting only two of the three exposure conditions together with sham was sound but

on the other hand led to a reduced power. Several other arguments such as the different gender distribution in the two groups are not very important because each subject served as his/her own control and comparison between groups was not important in this investigation. Other criticism was expressed against statistical analysis. No correction for multiple testing was applied. While some advice protection against inflation of type I error others recommend correction only for crucial experiments and not for pilot studies like this. Another, more serious, criticism was put forward against disregarding sequence of experimental conditions. As mentioned above, sequence, transfer, and time-of-day effects could have compromised results because such effects are not completely removed by balancing exposure sequence. Due to this criticism several studies were planned that should investigate whether the effects observed in the TNO study are robust and could be replicated under improved study designs.

One of these experiments was performed in Switzerland [21]. Like in the TNO study, two groups of individuals were included: one with self-reported sensitivity to RF-EMF (radio-frequency EMF) and a reference group without complaints. The first group consisted of 33 persons (19 females, 14 males) and the reference group of 84 persons (43 females, 41 males). The experiment consisted of three experimental and one training session each 1 week apart performed on the same time of day ( $\pm 2$  h). Design was a randomized double-blind cross-over design like in the case of the TNO study, however, with a week between sessions and with all subjects tested under all experimental conditions that were solely simulated UMTS base station exposure at 1 V/m, 10 V/m and sham. The same UMTS protocol as in the TNO study was used. Each exposure condition lasted for 45 min. During exposure two series of cognitive tasks were performed. After each exposure condition the same questionnaire as has been used in the TNO study was applied and questions about sleep in the previous night, alcohol, coffee consumption, etc., were asked. Moreover, subjects had to rate the perceived field strength of the previous exposure condition on a visual analogue scale. In addition, before and after each session the short Questionnaire on Current Disposition [22] was answered by participants. Questionnaires were presented in a separate office room.

Except for a significant reduction of performance speed of sensitive participants in the 1 V/m condition in one of six cognitive tests no effect of exposure was detected. In particular, no reduction of wellbeing neither as assessed by the TNO questionnaire nor from scores of the Questionnaire on Current Disposition was found. Also correlation between perceived and real exposure was not more often positive than expected from chance. Fig. 2 compares results of the TNO study and the results of Regel et al. [21] for the matching conditions (UMTS at 1 V/m). There are some notable differences between the two studies: first, the reference group in the study of Regel et al. [21] had significantly higher scores (reduced wellbeing) as the reference group in the TNO study in both the sham and the UMTS 1 V/m condition; second,

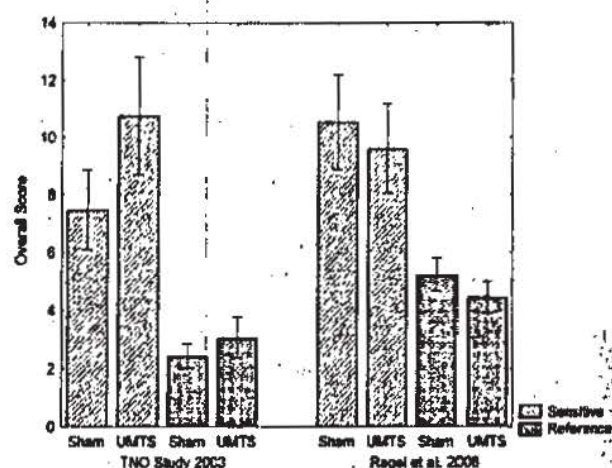


Fig. 2. Comparison of mean ( $\pm$ SEM) overall wellbeing scores (TNO questionnaire) obtained in the TNO study [19] and in the study of Regel et al. [21] for the matching conditions: Sham exposure and UMTS exposure at 1 V/m in sensitive participants and the reference group.

average scores from sensitive participants after exposure at 1 V/m are comparable in both studies but the sham condition resulted in much lower scores (better wellbeing) in the TNO study. There are several explanations for this difference between the two studies. It is possible that the reference group in the TNO study consisted of exceptionally robust individuals. The fraction of males was higher in the TNO study and males have typically lower scores. However, considering that the reference group in the TNO study was almost 10 years older (mean age 47 years) as compared to the study of Regel et al. [21] (mean age 38 years) this is not a satisfactory explanation. It is possible that the basic adversity of the experimental setup was higher in the latter study resulting in overall greater reduction of wellbeing. That this has not been observed in the sensitive group assumed to be more vulnerable to a 'nocebo' effect (the nocebo effect is the inverse of the placebo effect describing a situation when symptoms occur due to expecting adverse reactions) in both conditions could be due to a ceiling phenomenon. Although the study by Regel et al. [21] had an improved design and could not replicate the earlier findings of the TNO study, doubts exist whether this can be considered a refutation of an effect of UMTS exposure on wellbeing.

Another experimental study in sensitive and non-sensitive participants has been conducted in Essex, Great Britain, by Eltiti et al. [23]. The experiment consisted of two phases: an open provocation test and a series of double-blind tests. In the open provocation phase 56 self-reported sensitive and 120 non-sensitive control individuals participated. Of these, 44 sensitive (19 females, 25 males) and 115 controls (49 females, 66 males) also completed the double-blind tests. Participants took part in four separate sessions each at least 1 week apart. First session was the open provocation trial, sessions 2–4 were double-blind exposure trials with a sham, a GSM and a UMTS exposure condition. Double-blind sessions were reported to last for 1.5 h, however, Table 1 of the

article showed an overall length of 48 min only. GSM exposure was a simulated base station signal with both a 900 and a 1800 MHz component each at an average level of 5 mW/m<sup>2</sup> and with a simulated BCCH with all time slots occupied and a TCH with a simulated 40% call activity resulting in a total of 10 mW/m<sup>2</sup> GSM exposure at the position of the participants (corresponding to 1.9 V/m E-field strength). The UMTS signal had a frequency of 2020 MHz with a power flux density of 10 mW/m<sup>2</sup> over the area where the participant was seated. Traffic modeling for the UMTS signal was achieved using a test model representing a realistic traffic scenario, with high peak to average power changes. During double-blind sessions participants watched a BBC "Blue Planet" video for 20 min, performed a mental arithmetic task for 20 min, performed a series of cognitive tasks lasting 8 min, and made 'on/off' judgments. During the first 40 min every 5 min subjective wellbeing was recorded on visual analogue scales (VAS) measuring anxiety, tension, arousal, relaxation, discomfort, and fatigue. In addition a symptom scale consisting of 57 items was answered. During the whole period physiological measurements of heart rate, blood volume pulse, and skin conductance were performed.

Physiological measurements revealed higher average values for sensitive individuals compared to controls which were especially high under UMTS exposure conditions. Symptom list did not reveal any differences between double-blind conditions, but the overall frequency of solicited symptoms was low. Concerning subjective wellbeing as assessed by VAS there were increased values for anxiety, tension, and arousal under GSM and especially UMTS exposure conditions. Combining all scores of the six scales (with relaxation reflected) reveals a significant increase during UMTS exposure compared to sham for the sensitive group and a significant reduction for the control group (see Fig. 3). Judgment of participants about presence of exposure was not correct more often than inferred from chance.

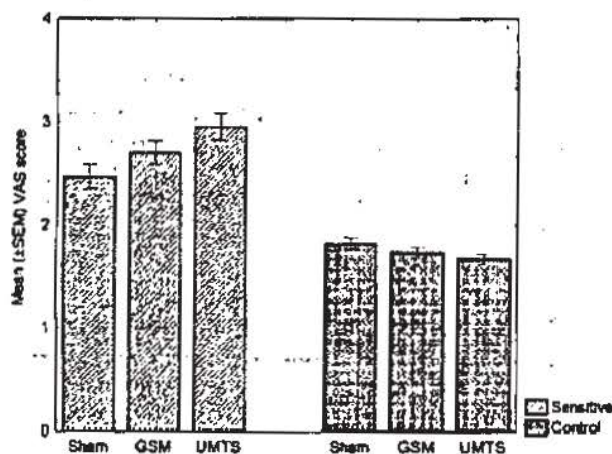


Fig. 3. Mean (±SEM) total visual analogue scale scores computed from Table 2 of Eltiti et al. [23] during sham, GSM, or UMTS exposure in sensitive and control individuals.

The increased values for anxiety, tension, and arousal found in this investigation were interpreted by the authors as due to an imbalance in the sequence of conditions with UMTS being more often the first exposure condition presented in the double-blind sessions. The imbalance was due to not reaching the predefined sample size. This points to the importance of setting the block size for randomization to a low level (e.g. in this experiment with 6 possible exposure sequences a block size of 18 would have been appropriate). Interpretation of authors, however, is questionable as pointed out by Rösli and Huss [24]. For arousal tabulated values stratified for sequence of presentation (Table 3 in [23]) demonstrates that the difference between sham and UMTS is present regardless of sequence of presentation. An additional analysis of the authors presented in response to the criticism in their statistical analysis seems to support their view that the observed difference to sham is due to a sequence effect. However, it seems that this analysis has not been correctly applied as the sequence was introduced as a between subjects factor which corrects only the interaction between group and condition. Also the figure they provided [23] is inconclusive as it only demonstrates what is already known: that first exposure leads to higher reduction of wellbeing (higher values of arousal). This investigation, although well designed and applying a more realistic exposure scenario than the other two studies, leaves some questions open. Despite an apparent corroboration of the findings of the TNO study, the imbalance in the sequence of exposures makes it difficult to decide whether the interpretation of authors that the observed effect is due to an excess number of UMTS exposures presented first in the sequence is correct or an actual effect occurred. Irrespective of these difficulties, consistent with the other investigations, wellbeing was not strongly affected.

There are several other investigations of a similar type that have been completed and already reported at scientific meetings (e.g. Watanabe, Japan; Augner, Austria, personal communication) but have not yet been published.

### 3.2. Animal and in vitro experiments

Anane et al. [25] applied the DMBA (7,12-dimethylbenz(a)anthracene) model of mammary tumor induction in female Sprague-Dawley rats to test whether a sub-chronic exposure to microwaves from a GSM-900 base station antenna affects tumor promotion or progression. Exposure was 2 h/day, 5 days/week for 9 weeks starting 10 days after application of 10 mg DMBA administered at an age of animals of 55 days. Exposure was applied in an anechoic chamber with animals placed in Plexiglas compartments that confined animals to a position parallel to the E-field. Details of the exposure protocol were not provided. Two series of experiments were conducted with four groups of 16 animals each. In the first experiment groups were: sham, 1.4, 2.2, and 3.5 W/kg whole-body SAR, and the second experiment with sham, 0.1, 0.7, and 1.4 W/kg. In the first experiment the tumor incidence rate was significantly increased at 1.4